Original Article

Virtual Screening To Identify Protein Targets In Aggregatibacter Actinomycetemcomitans Interacting With Berberine

Shruthi Devi R[1], Dr. Jeevitha.M[2*], Dr. Vijayashree Priyadharsini J[3]

[1]Saveetha Dental college and hospitals Saveetha Institute of medical and technical sciences Saveetha university, chennai-77,Tamilnadu, India.

[2]Senior lecturer, Department of Periodontics Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai -77, India

[3]Associate Professor, Clinical Genetics Lab, Cellular and Molecular Biology research center, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai -77, India

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Abstract

Introduction: Oral cavity hosts innumerable numbers of microorganisms which are associated with disease such as dental caries, periodontal and deep-seated infections. The resistance developed by these organisms against synthetic drugs have spurred interest towards using phytocompounds as an alternative to combat diseases caused by dental pathogens. *Aggregatibacter actinomycetemcomitans* is one such oral and systemic pathogen associated with aggressive forms of periodontitis and with endocarditis causing chronic localized infections.

Aim: To virtually screen and identify the protein targets of *A. actinomycetemcomitans* interacting with berberine.

Materials and Methods: Computational tools such as STITCH v5.0 (16), VICMPred (17), VirulentPred (18), BepiPred v2.0 (19, 20) and pSORTb (21) were used to identify potential targets of *A. actinomycetemcomitans* interacting with the phytocompound berberine and the results obtained were analyzed

Results: The proteins 3-deoxy-D-manno-octulosonic-acid kinase, MerR family transcriptional regulator, nucleoid occlusion protein and DNA-binding transcriptional repressor FabR were found to be virulent. These proteins were either involved in the process of metabolism or cellular process.

Conclusion: The study identified molecular targets of berberine in *A. actinomycetemcomitans* which have to be further validated through experimental procedures to identify the effect of this drug in a physiological environment.

Key words: Aggregatibacter actinomycetemcomitans, Berberine, Protein targets, Virtual screening

Introduction

The most common cause of tooth loss considered world wide is the destruction of the periodontium due to the periodontitis. It arises when there is a chronic inflammation of gingiva or a progressive destruction of alveolar bone or the supporting tissue(1).It is caused by a bacteria which is epidemiologically associated with various systemic diseases like the cardiovascular disease and rheumatoid arthritis that occurs due to the subgingival bacterial composition of the dental plaque.Recently clinical and epidemiological studies revealed that it is more prominent in individuals with rheumatoid arthritis as it could be one of the reason in initiating and maintaining autoimmune inflammatory response that is involved in causing it. Hence it was found that microbes alone are not the sole reason for causing periodontitis(2) .The disease may also arise from a community based attack on the host(3) .Apart from this, smoking,stress,diet, proper oral hygiene and other social factors may also lead to this disease(4).The International Workshop for Classification of Periodontal Diseases and Conditions in 1999 has described the aggressive periodontitis as a "multifactorial, severe and rapidly progressive form of Periodontitis, which primarily but not exclusively affects younger patients.

The most common organism that is said to cause this aggressive periodontitis is the *Aggregatibacter actinomycetemcomitans,* which is a systemic gram negative,capnophilic and coccobacillus bacteria(5).It is also found to be associated with non - oral infections like endocarditis(6)The prevalence of the bacteria varies based upon the geographic origin, age and the lifestyle of the population.There are seven serotypes that have genetically different lineages that are recognised by an immunodominant antigen which is O polysaccharide that is present in its liposaccharide. It usually inhabits the oral mucosa and colonizes as a facultative intracellular pathogen and

slowly moves towards the sub gingival crevices(7).It produces a variety of virulence factors and the most important one among them is the cytolethal distending toxin (Cdt) and leucotoxin type A.The former cause destruction of cell by inhibiting their proliferation while the latter is a type of RTX toxin that selectively affects the human cells that is of haematopoietic in origin by binding to the lymphocyte function associated receptor (LFA-1) that causes the disruption of the membrane integrity by either producing pores or through the rapid influx of Ca2+ ions that result in either apoptosis or necrosis(8) . The mechanism of CDT is that it kills the host cells by inducing apoptosis of lymphocytes through the DNAse activity and killing of gingival fibroblast and periodontal ligament cells by inhibiting their proliferation process simultaneously thereby causing a growth arrest in both G1 and G2/M phase and enlarged periodontal connective tissues(9)RTX leukotoxin targets only neutrophils and monocytes and its action is influenced by a novel type IV secretion system that is involved in bacterial adhesion. The chaperonin 60 secreted by it has potent bone resorbing activities has The other virulence factors include lipopolysaccharides, Surface-associated material, chemotactic inhibition factors, proteases, collagenases etc.Hence, production of effective inhibitors against this leucotoxin and CDT can prevent the periodontitis disease caused by it(10).

New drugs can be designed through a traditional receptor based virtual screening which helps in the discovery of various bioactive compounds that bind with the target protein because,the main principle behind the mechanism of these drugs and bioactive compounds is their interaction with the protein targets(11). Hence, target identification of these proteins plays an important role in the field of biomedical research and discovery of drugs as it helps to understand the mechanism of the drugs and also show the potential therapeutic applications,its use and adverse side effects of the drug (12) .The virtual screening (VS) is considered to be an important component of the computer based search for the discovery of novel compounds.This can be approached by 2 methods, one is by docking that requires a 3D structure knowledge of the target binding site and the other one is the similarity based VS that uses one or more compounds that bind with the protein as structural query.it is a type of receptor based screening and considered as a reliable and inexpensive way of identifying recently (13) .

Berberine (BBR) is considered as a naturally occurring alkaloid that is derived from *Berberis vulgaris* (Barberry), *Mahonia aquifolium* (Oregon grape), *Berberis aristata* (tree turmeric), *Hydrastis canadensis* (goldenseal) etc. It was earlier used in the treatment of bacterial diarrhea, anti-infection, and ocular trachoma infections in China. Apart from that, it also exhibits anticancer activity against melanomas, leukemia,hepatocellular carcinoma, multiple myelomas etc. It plays an important role in modulating protein degradation(14) .It activates an E3 ubiquitin ligase Cbl to degrade EGFR protein, which leads to the inhibition of proliferation of cell both in mouse and human colon cancer cells (15). Previous study has reported that the BBR downregulates the miR-21 expression through IL6/STAT3 in MM cell lines, thereby leading to the inhibition of secretion of IL-6 (16).

Hence the present study aims to virtually screen and identify the protein targets in *A. actinomycetemcomitans* on interacting with berberine so that it can prevent periodontitis as only very few studies are based on using berberine protein

Materials and Methods

Study Design

Our present study follows an observational study design as it screens and identifies protein interactions of berberine with the bacteria virtually. Softwares like STITCH database, Virulent Pred,VCIM Pred etc are used.

In silico tools have immensely cut-down the time and cost required for screening phytocompounds. The following computational tools were used for this present study:

1. STITCH v5.0 - Identification of drug protein interaction
2. VICM Pred - Identified of functional and class of protein
3. VirulentPred - identification of virulent nature of protein BepiPred v2.0 - Identification of B cell epitope from virulent protein sequence
4. PSORTBv3.0 - Identification of the subcellular location of the virulent proteins

These Computational tools such as STITCH v5.0 , VICMPred, VirulentPred, BepiPred v2.0 and pSORTb were used as it identifies the potential targets of *Aggregatibacter actinomycetemcomitans* that interacts with the phytocompound berberine.

Predicting protein drug interaction

The STITCH database includes strains as it is known to provide a huge amount of information about the interaction between the drug and the protein both physically and functionally aggregated from primary databases (17) .

Prediction of Virulence factor

The virulent Pred tool classifies the protein as virulent or avirulent based on the presence of amino acid present in it. Whereas VCIM Pred tool classifies the protein into four groups as
Proteins involved in Cellular process information storage, metabolism and virulence (18) (19)**.**

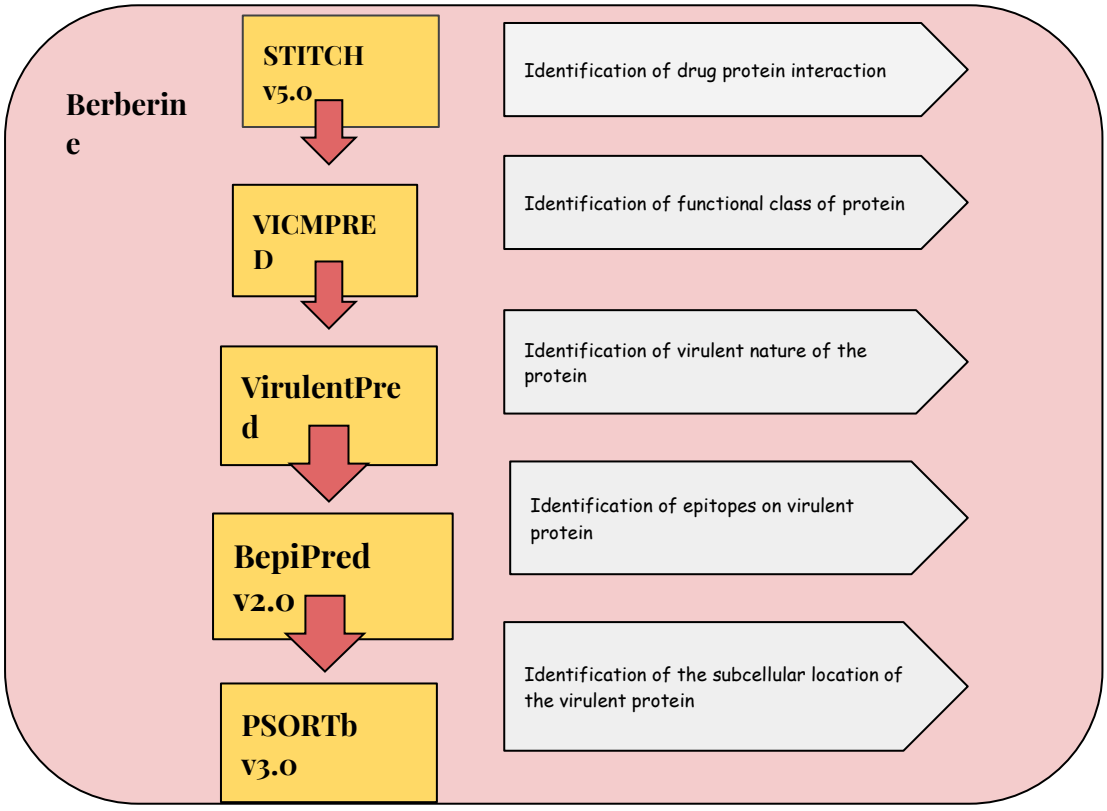
Epitope prediction

To be a part of epitope, the score should be above the threshold value (0.5). It helps in preparing local drug targets (20,21).

Subcellular localisation prediction

PSORTBv3.0 is an algorithm type that assigns a localized site from the amino acid sequence to the protein (22).

The schematic representation of the procedure done for screening the targets present in *A. actinomycetemcomitans* is shown below and the results obtained were further analyzed.



Results and Discussion

Berberine, a plant alkaloid, is considered to show a potent antimicrobial activity against selected oral pathogens. A study by Xie and team demonstrated that berberine was found to be more effective irrigant than saline against selected endodontic pathogens in vitro and when combined with chlorhexidine produced good bacterial efficacy a NaOCl(22).It is also used as a potential medicine for treating bone disorders, hence it can be proposed to be used in the root canals to enhance the repair of roots in immature teeth with apical periodontitis. By using BBR, more tissues are formed, with longer and thicker root walls with smaller apex diameters. It also induces beta catenin expression (23). Another study investigated the effect of BBR and the test of essential oils on oral pathogens. It was found that the combination of BBR and EOs inhibited the growth of *P. gingivalis*, *F. nucleatum* and *A. actinomycetemcomitans* at MICs ranging from 15.6 to 125 µg/ml for BBR. The BBR/Methyl salicylate

(15.6 µg/ml/ 0.03 %) combination produced a synergistic growth inhibition against *A. actinomycetemcomitans* (FICI = 0.375)(24).

A. actinomycetemcomitans is considered as an oral and systemic pathogen associated with aggressive forms of both periodontitis and subacute infective endocarditis. A gene has been identified in *A. actinomycetemcomitans* which is required for LtxA secretion and antimicrobial resistance(25).It has been observed from the present study that berberine could target several virulent proteins of *A. actinomycetemcomitans,* which plays crucial roles in the cellular and metabolic pathways. This is the first study performed to identify potential protein targets based on virtual screening of *A. actinomycetemcomitans* against berberine. Various other studies have also been done on this topic using caffeine, reserpine, theophylline etc.

Berberine was found to interact with numerous proteins of *A. actinomycetemcomitans.* Among all the interacting proteins, four proteins *viz.,* 3-deoxy D-manno-Octulosonic acid kinase, MerR family transcriptional regulator, nucleoid occlusion protein, DNA-binding transcriptional repressor FabR were found to be virulent as predicted by VirulentPred. The subcellular location of the proteins were further assessed using the pSORTb tool, which demonstrated the presence of 3-deoxy D-manno-Octulosonic acid kinase in the cytoplasmic membrane and nucleoid occlusion protein in the cytoplasm. The subcellular location of other 2 proteins did not return any value for interpretation. Epitopes are antigenic determinants that can elicit an immune response in the host. Hence the search for epitopes on virulent proteins using the BepiPred tool was conducted. Among the four proteins, DNA-binding transcriptional repressor FabR was found to have more than 10 epitopes. The present study provides insight into the protein-drug interaction that can present as an antimicrobial effect exerted by Berberine.

Hence berberine can be used as an alternative to synthetic compounds. But there are certain limitations in the study: Bonding between the compound and the protein of the pathogen may purely be a physical interaction that might not result in a functional interaction. Berberine induced interactions may not be the same in complex biological environments and proteins of the bacteria may mimic the host proteins which may end up causing side-effects.

Conclusion

The study identified molecular targets of berberine on the bacteria *Aggregatibacter actinomycetemcomitans* which should be further validated to confirm the critical pathway triggered by the phytocompound under physiological conditions.

Authors contributions

Shruthi Devi R : Literature search, data collection, analysis, manuscript drafting.
Dr.Jeevitha: Aided in conception of the topic, has participated in the study design, statistical analysis and has supervised in preparation and final corrections of the manuscript.
Dr.Vijayashree Priyadharshini J : Aided in conception of the topic, has participated in the study design, statistical analysis and has supervised in preparation and final corrections of the manuscript.

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Conflict of interest

The author declares that there was no conflict of interest in the present study.

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Table 1: Proteins of Aggregatibacter actinomycetemcomitans interacting with berberine A

Organism	Identifier	Proteins which interacts with berberine A	VICMPred Functional Class	Virulent Pred	Virulent Pred Score
Aggregatibacter actinomycetemcomitans	D7S_1738	MerR family transcriptional regulator	Metabolism	Avirulent	-1.108
	D7S_1516	Cell division protein FtsZ	Cellular process	Avirulent	-1.024
	D7S_1144	SoxR protein	Cellular process	Avirulent	-1.118
	D7S_0879	3-deoxy-D-manno-octulosonic-acid kinase	Metabolism	Virulent	0.5992
	D7S_1097	AcrA protein	Virulence factor	Avirulent	-1.119
	D7S_1168	MerR family transcriptional regulator	Cellular process	Virulent	0.7466
	D7S_1117	Nucleoid occlusion protein	Cellular process	Virulent	0.4617
	D7S_1020	carboxylesterase type B	Metabolism	Avirulent	-1.042
	D7S_0057	AcrR protein	Metabolism	Avirulent	-0.023
	D7S_1246	DNA-binding transcriptional repressor FabR	Metabolism	Virulent	0.5479

Table 2: The subcellular localization of virulent proteins identified in dental pathogens

Proteins which interacts with berberine A	Subcellular location of the protein	Score
3-deoxy-D-manno-octulosonic-acid kinase	Cytoplasmic membrane	7.88
MerR family transcriptional regulator	Unknown	-
Nucleoid occlusion protein	Cytoplasmic	9.97
DNA-binding transcriptional repressor FabR.	Unknown	-

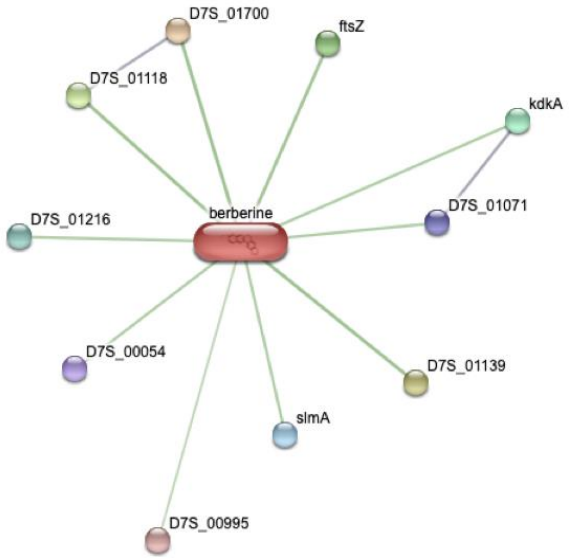
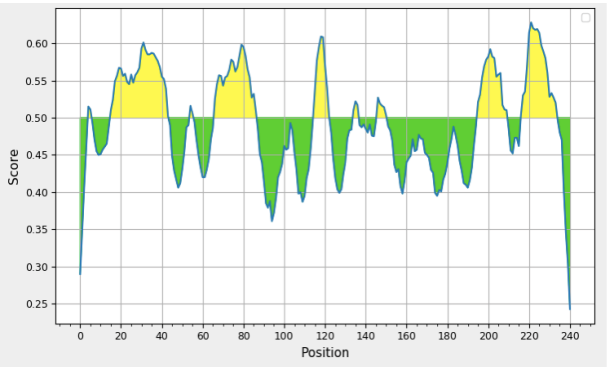


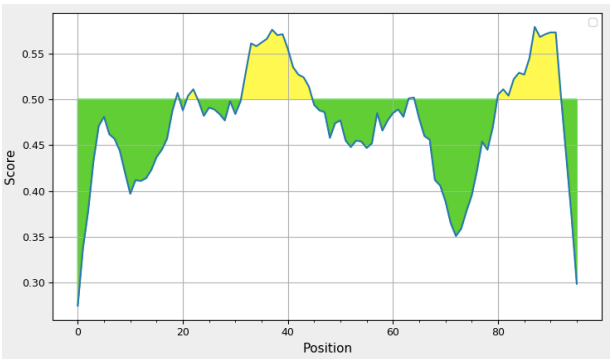
Figure 1: Protein interaction network of Aggregatibacter actinomycetemcomitans with berberine A

(A)



Predicted peptides:

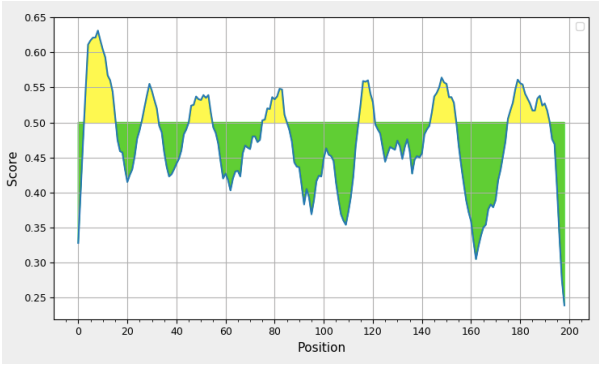
No.	Start	End	Peptide	Length
1	5	6	QL	2
2	16	44	DQPLANQTQFFEAAFWQQQNRVIGAAKGR	29
3	55	56	LF	2
4	67	87	RGGLWGKINKDRYHFSELKNT	21
5	116	123	KGNLGMCY	8
6	135	137	ARD	3
7	147	151	LESTQ	5
8	196	210	CGEKSGRFWKEANLQ	15
9	217	234	NKEAARMHIHFTEQNWQD	18



Predicted peptides:

No.	Start	End	Peptide	Length
1	20	20	G	1
2	22	23	RQ	2
3	33	45	IISVEGHPEQAVF	13
4	64	65	EA	2
5	81	93	EELRKQTRSISLL	13

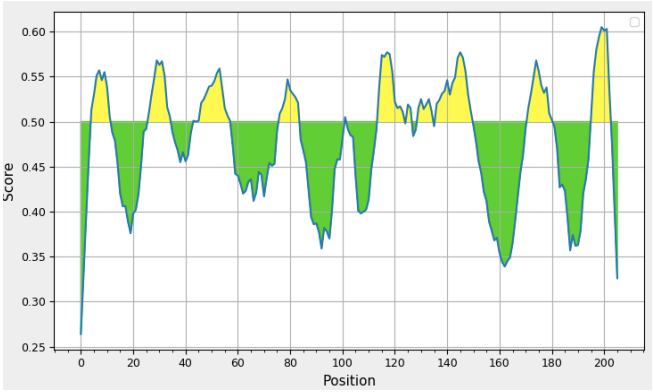
(B)



Predicted peptides:

No.	Start	End	Peptide	Length
1	4	16	ANKRSIKERRQQV	13
2	27	33	ERGMERM	7
3	47	55	AALYRYYPS	9
4	76	85	SQSIKHETNT	10
5	116	121	MFEDAK	6
6	145	155	KLREGRGFNVD	11
7	176	193	RSNFRHMPNQGFNQQWQL	18

(C)



Predicted peptides:

No.	Start	End	Peptide	Length
1	5	12	RAQQKEKT	8
2	27	35	EKSFSNLSL	9
3	44	58	IAPTSFYRHFRDMNE	15
4	77	84	QARKRIAN	8
5	102	102	N	1
6	115	124	SGTSQAFRTA	10
7	126	127	AR	2
8	130	135	KHFIDE	6
9	137	150	SEYIAHKNHYSQYI	14
10	172	181	MSKTEREQLK	10
11	196	203	KYETRDKY	8

(D)

Figure 2: Epitope prediction (A) 3-deoxy-D-manno-octulosonic-acid kinase (B) MerR family transcriptional regulator (C) Nucleoid occlusion protein and (D) DNA-binding transcriptional repressor FabR.