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VIRTUAL SCREENING TO IDENTIFY PROTEIN TARGETS IN AGGREGATIBACTER ACTINOMYCETEMCOMITANS INTERACTING WITH CAFFEINE.

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ABSTRACT

INTRODUCTION

Aggregatibacter actinomycetemcomitans is a perio-pathogenic bacteria that has long been associated with severe periodontitis. The mechanisms of its pathogenicity have been studied in humans and pre-clinical experimental models. The aim of the present study was to identify the protein targets in *Aggregatibacter actinomycetemcomitans* interacting with caffeine through virtual screening.

MATERIALS AND METHODS

Computational tools were used to identify the targets, assess its functional role and virulence property. The STITCH tool was used for identifying the proteins of A.actinomycetemcomitans interacting with caffeine. Further, the peptide epitopes present in the virulence factors were identified using the BepiPred tool.

RESULTS

Multiple proteins of *A. actinomycetamcomitans* were found to interact with caffeine, of which only thiol disulphide interchange protein was identified as a virulence factor. Several epitopes have been identified in the protein. The

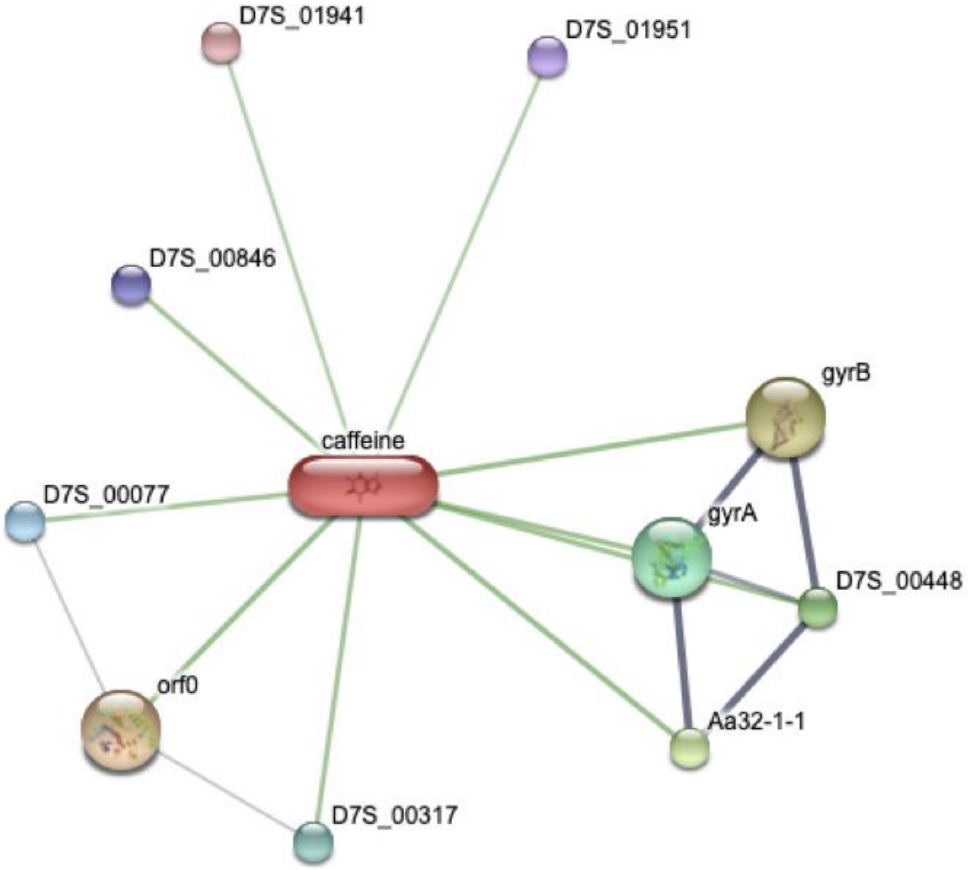
CONCLUSION

The present in silico study provides preliminary data on the protein targets and its virulence factor of *Aggregatibacter actinomycetemcomitans* interacting with caffeine.

KEY WORDS

Caffeine, virulence factor, Aggregatibacter actinomycetamcomitans,periodontitis, protein target, Innovative technique.

GRAPHICAL ABSTRACT



Protein interaction network of Aggregatibacter actinomycetemcomitans with caffeine

INTRODUCTION:

Aggregatibacter actinomycetemcomitans is an oral and systemic pathogen associated with aggressive forms of periodontitis. Periodontal diseases are characterized by chronic inflammation of the gingiva, and progressive destruction of alveolar bone and supporting tissues around the teeth resulting in tooth loss. Colonization by the Gram-negative human pathogen *Aggregatibacter actinomycetemcomitans* is strongly associated with aggressive forms of periodontitis in adolescents and young adults (Haubek *et al.*, 2008)(Henderson, Ward and Ready, 2010; Johansson and DiRienzo, 2021). Different serotypes of A. actinomycetemcomitans have differential virulence factor ex - catabolic effects on periodontal tissue homeostasis(Herbert, Novince and Kirkwood, 2016). Coffee is one of the most widely consumed beverages in the world. Hence, understanding its composition and actions on the human body are of scientific benefit. Coffee bean extract has been known to have antimicrobial effects against both Gram-positive and Gram-negative bacteria

as far back as 1989 (Toda *et al.*, 1989; Herbert, Novince and Kirkwood, 2016). Some components in coffee such as caffeine, volatile and non-volatile organic acids, phenols and aromatic compounds are reported to have antimicrobial activity. Chlorogenic acid (CGA) and caffeic acid, which are non - volatile organic acids found in coffee, inhibit the growth of some Gram-positive microorganisms such as *Staphylococcus aureus, Bacillus cereus, Lactobacillus bulgaricus, Streptococcus lactis, Streptococcus faecalis and Gram-negative bacteria such as E.coli, Salmonella typhi* and *Pseudomonas aeruginosa(Gloria, Almeida and Engeseth, 2019)*. Caffeine has demonstrated significant antibacterial properties against the cariogenic bacteria Streptococcus mutans and streptococcus militants and has also been found to be effective against the periodontal pathogens *P. gingivalis* and *P. intermedia*, as well as *Candida albicans (Mehta et al., 2014)*. However, there is not much evidence on the antimicrobial activity of caffeine against *Aggregatibacter actinomycetemcomitans*. Our team has extensive knowledge and research experience that has translated into high quality publication (Alsubait *et al.*, 2018; Del Fabbro *et al.*, 2018; Ramesh *et al.*, 2018; S *et al.*, 2018; Vellappally, Al Kheraif, *et al.*, 2018; Venkatesan, Rekha, *et al.*, 2018; Venkatesan, Singh, *et al.*, 2018; Jayaseelan and Arumugam, 2019; Varghese, Ramesh and Veeraiyan, 2019; Vellappally, Al Kheraif, Anil, *et al.*, 2019; Vellappally, Al Kheraif, Divakar, *et al.*, 2019; Paramasivam and Vijayashree Priyadharsini, 2020; Paramasivam *et al.*, 2020),(Ezhilarasan *et al.*, 2021; Kavarthapu and Gurumoorthy, 2021; PradeepKumar *et al.*, 2021; R *et al.*, 2021; Sarode *et al.*, 2021) (Vellappally, Abdullah Al-Kheraif, *et al.*, 2018) (Aldhuwayhi *et al.*, 2021). Hence, the rationale of this study is to identify the protein targets in Aggregatibacter actinomycetemcomitans that interact with caffeine.

MATERIALS AND METHODS

STUDY DESIGN

The present study followed an observational study design which aimed to screen for those proteins or virulence factors of *Aggregatibacter actinomycetemcomitans* which could possibly interact with caffeine. The interaction of drugs with proteome of bacteria were analyzed using STITCH v.5 pipeline12 and the virulence properties of the interacting proteins were deduced by VICMPred13 and VirulentPred softwares.

PREDICTION OF PROTEIN - DRUG INTERACTIONS

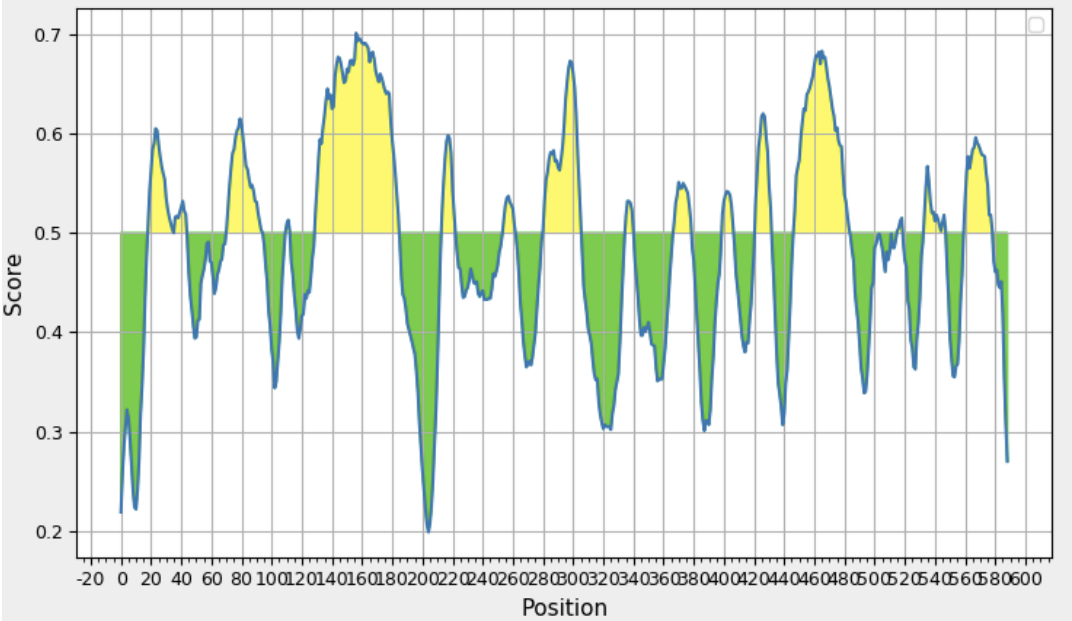
STITCH database (Version 5; 2016) provides an exhaustive platform for known and predicted interactions between chemicals and proteins. The interactions include direct or physical and indirect or functional associations which stem from computational prediction and from interactions aggregated from other (primary databases). The repertoire of proteins which interacts with *A. actinomycetemcomitans* were further used for predicting virulence (Garg and Gupta, 2008; Szklarczyk *et al.*, 2016).

VIRULENCE PREDICTION

VICMpred (Saha and Raghava, 2006) and VirulentPred pipelines were used for the identification of virulence factors targeted by *A. actinomycetemcomitans*. These tools employed a support vector machine (SVM)-based five-fold cross-validation process to validate results. Virulence factors were screened based on amino acid composition using the Virulent Pred tool which classified them into two groups, that is, virulent and avirulent. VICMpred groups proteins into four major classes, namely, proteins involved in cellular process, metabolism, information storage, and virulence. The overall accuracy of VICMpred and VirulentPred servers were 70.75% and 86%, respectively. The FASTA (Fast - All) format of the proteins retrieved from the NCBI database were used as an input to run the algorithm (Yu *et al.*, 2010).

PREDICTION OF B - CELL EPITOPES IN THE VIRULENT PROTEINS

The BepiPred-2.0 server predicts B-cell epitopes from a protein sequence, using a Random Forest algorithm based on epitopes and non-epitope amino acids determined from crystal structures. The residues with scores above the threshold (>0.5) are predicted to be part of an epitope and colored in yellow on the graph . (Fig 1)



Predicted peptides:

No.	Start	End	Peptide	Length
1	19	44	AVDLFNQKPKFLPVDQAFQLQAEQYG	26
2	71	94	QPIESQPQFLSRAEQYEDPYFGTV	24
3	110	113	SKPQ	4
4	130	185	PPKTKQFMLGELPHNAVETSLAAQEETKSAVRNPSVFSSQQNQLADSLFQSKYAML	56
5	214	222	NRTEHSHTW	9
6	255	262	VALQSPYV	8
7	281	306	FTLQLPPSLQTKLTQLSQQQKRGAFF	26
8	336	341	QSGDLV	6
9	368	379	ILPKSGAWMENV	12
10	400	407	PLDWEPRL	8
11	422	432	QMRTNGIGLLF	11
12	448	484	QNWLWQNNIDHARTHSAVKNSLEFQSVQSYEELQLVL	37
13	516	519	QVQD	4
14	534	548	NSENNRTLMKQLSIT	15
15	561	579	KEISSHRITGFMEGEAFLQ	19

Figure 1: Epitope prediction of the virulent protein thiol-disulfide interchange protein

RESULTS

The STITCH pipeline was used to identify the protein interaction between *A. actinomycetemcomitans* and caffeine (Fig. 1). Further each of the proteins interacting with the

drugs was assessed for their virulence property using VirulentPred and VICMpred. The scores produced by these algorithms confirmed the nature of the proteins and grouped them into two classes i.e., virulent and avirulent. Caffeine was found to react with a plethora of proteins in *A. actinomycetemcomitans* (Table 1).

Majority of the proteins interacting with caffeine were found to have metabolic function, while few proteins like Exonuclease III and DNA gyrase A have cellular process and glycogen synthase has virulence factor. Interestingly, the scores from VirulentPred marked only thiol disulphide interchange protein from *A. actinomycetamcomitans* as a virulence factor. All the other proteins analysed were found to be avirulent.

Table 1: Proteins of *Aggregatibacter actinomycetemcomitans* interacting with caffeine

ORGANISM	IDENTIFIER	PROTEINS WHICH INTERACT WITH CAFFEINE	VICM PRED FUNCTIONAL CLASS	VIRULENT PRED	VIRULENT PRED SCORE
Aggregatibacter actinomycetemcomitans	D7S _1988	Thiol disulphide Interchange protein	Metabolism	Virulent	0.7997
	D7S_1999	Thiol disulphide Interchange protein	Metabolism	Avirulent	-0.992
	D7S_0865	Glycerophosphoryl Diester Phosphodiesterase	Metabolism	Avirulent	-0.881
	D7S_0081	Glycogen synthase	Virulence factor	Avirulent	-1.064

	D7S_2258	Exonuclease III	Cellular process	Avirulent	-1.005
	D7S_0326	Maltodextrin phosphorylase	Metabolism	Avirulent	-1.022
	D7S_0458	DNA Topisomerase IV Subunit A	Metabolism	Avirulent	-1.040
	D7S_0455	DNA Topisomerase IV Subunit B	Metabolism	Avirulent	-1.032
	D7S_0170	DNA gyrase A	Cellular process	Avirulent	-1.012
	D7S_0903	DNA gyrase subunit B	Metabolism	Avirulent	-1.019

DISCUSSION

In silico validation is inevitable while choosing a compound or drug to be tested under in vitro or in vivo conditions. In addition to cutting down the cost involved in conducting experiments, it provides clues about the specific mechanism or pathways which can be targeted during preliminary screening which could make the process less time consuming and more focused. The present study has been designed to identify potential interactions, especially the virulence factors of *A. actinomycetemcomitans* with caffeine.

A. actinomycetemcomitans is a Gram negative capnophilic coccobacillus, which is commonly isolated from the oral cavity of adolescents and young adults afflicted by aggressive periodontal disease states. Elimination of this pathogen plays an essential role in treatment of aggressive periodontitis. Caffeine is known for its antibacterial property. Caffeine inhibits syntheses of

proteins and DNA by inhibiting the incorporation of adenine and thymine. Caffeine also enhances genotoxicity after DNA damage (Roberts, 2013). This study reveals the virulence of the proteins in *A.actinomycetemcomitans* while interacting with caffeine. The protein thiol disulphide interchange protein is the only protein which shows virulence factor. This helps in gaining a better understanding on the pathogenicity of *A.actinomycetemcomitans* and it's interaction with caffeine.Several studies have provided substantial evidence on the antibacterial effect of caffeine against various pathogens. Khan et al., demonstrated that caffeine loaded onto nanoparticles exhibited anti-biofilm, antibacterial and extended effects against persisting gram positive and gram negative organisms. (Khan *et al.*, 2021) Chakraborty et al., stated that caffeine along with its biofilm inhibition had the ability to reduce the secretion of virulence factors from Pseudomonas aeruginosa. (Chakraborty *et al.*, 2020) stated that caffeine demonstrated significant antibacterial activity against selected bacterial isolates however, its combination with the selected antibiotics resulted in significant antagonistic interactions. (Olajuyigbe *et al.*, 2017) Sledz et al., demonstrated that caffeine is a potential tool for the control of diseases caused by plant - pathogenic bacteria. (Sledz *et al.*, 2015)(Gupta *et al.*, 2014)

Although the in silico tools employed provides preliminary data on the underlying molecular interaction between the compound and protein network of red complex pathogens, there exists some limitations in the study viz., (a) the bonding between the compound and the protein of pathogen may purely be a physical interaction which may not reflect the functional role, (b) the drug induced interactions may not be the same in a complex biological environment and (c) the proteins of the red complex bacteria targeted by the compound might mimic host proteins. So to avoid the undesirable interactions of caffeine with host proteins, it is imperative to conduct in vitro and in vivo experiments, to gain clarity over the use of phytocompounds on human hosts without any adverse effects.

CONCLUSION

This study reveals that the thiol disulphide interchange protein in *A. actinomycetemcomitans* is virulent when interacting with caffeine. This study also provides evidence that caffeine can be a potent antimicrobial agent against *A. actinomycetamcomitans.*

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CONFLICT OF INTEREST

The authors declare that there were no conflicts of interest in the present study.

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