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AN INSILICO APPROACH TOWARDS IDENTIFICATION OF VIRULENCE FACTORS IN FUSOBACTERIUM NUCLEATUM TARGETED BY TRICLOSAN

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ABSTRACT:

Background: Fusobacterium nucleatum is a periodontal pathogen and an anaerobic oral commensal that has been linked to a variety of human disorders. Triclosan has been used in toothpastes, cosmetics and antiseptic soaps which are all examples of antibacterial and antifungal agents.

Aim: The aim of this study was to identify the virulence factors in Fusobacterium nucleatum targeted by triclosan through an insilico approach.

Materials and Methods: The STITCH v.5 pipeline12 was used to investigate drug interactions with the proteome of Fusobacterium nucleatum. VirulentPred and VICMPred were utilised to determine the virulence characteristic and functional class of the proteins using the pipeline.

Results: Fusobacterium nucleatum interact with proteins which involved in cellular processes, metabolism and pathogenicity were discovered to interact with it.

Conclusion: A thorough understanding of the interactions between these medications and their antibacterial agents would increase the list of advantages these treatments have in therapeutic situations. To validate the true interactions between medicines and pathogen protein repertoires, more in vitro study on a wide spectrum of infections is required.

Keywords: Fusobacterium nucleatum, Triclosan, Proteins

INTRODUCTION:

Fusobacterium nucleatum is an early and late colonisers of oral biofilm which co-aggregate with significant types of bacteria.(1) The bacterial species Fusobacterium nucleatum co-aggregates with early and late colonisers of oral biofilms.(2) F. nucleatum prevalence rises as illness severity, inflammation progresses, and pocket depth increases.(3) Environmental factors influence the abundance of F. nucleatum. Smoking promotes periodontal abundance in both healthy and sick people. Patients who diagnosed with uncontrolled type-2 diabetes and those with chronic periodontitis have higher levels of Fusobacterium nucleatum.(4) It is linked to gingivitis, which is reversible and advanced irreversible periodontitis, such as chronic periodontitis, localised aggressive periodontitis and generalised aggressive periodontitis.(5) Although it is an anaerobic bacterium, it tolerates oxygen in biofilm.

Triclosan is a fatty acid synthase inhibitor that can be found in various of product lines, notably body washes, soaps, mouth rinses and tooth pastes.(6) Due to its widespread use, triclosan can be found in a number of natural and man-made environments, includes surface water, wastewater, soil, drinking water, sewage treatment plants, organic wastes, landfills, and sediments.(7) According to new research, triclosan inhibits fat formation by suppressing the enzyme enoyl-acyl carrier protein reductase (ENR).(8) Despite its ubiquitous use in hygiene products, accumulating evidence suggests that triclosan interferes with hormone regulation.(9) Therefore, the present study was done as an insilico approach towards identification of virulence factors in factors in Fusobacterium nucleatum targeted by triclosan.

MATERIALS AND METHODS:

Study design: Our current research uses an empirical study design to look for a nature of proteins or category of virulency in Fusobacterium nucleatum that may interact with triclosan. The STITCH v.5 pipeline was used to evaluate drug interactions with the Fusobacterium nucleatum proteome, and VICMPred and VirulentPred softwares were used to identify the virulence features of the targeted proteins.

Prediction of protein-drug interactions: The STITCH database (Version 5; 2016) claims a comprehensive programme for known and projected chemical-protein interconnection. The interactions are from computational divination and interactions collected from other (primary) datasets, and they can be direct or indirect or functional. The protein repertoire that interacts with Fusobacterium nucleatum was also utilised to predict virulence.

Virulence prediction: The pipelines VICMpred and VirulentPred were used to identify triclosan-targeted virulence factors in Fusobacterium nucleatum. To validate the results, these tools used a support vector machine (SVM)-based five-fold cross-validation method. The VirulentPred tool was used to screen virulence factors based on amino acid composition, which is divided into two groups: virulent and avirulent. Proteins important in cellular processes, metabolism, information storage and pathogenicity are divided into four categories by VICMpred. The programme was conducted using the FASTA format of proteins acquired from the NCBI database as input.

Prediction of subcellular determination of proteins: Computational prognostications of protein subcellular limits aids in the development of new therapeutic targets or the validation of an antibacterial medication that targets the pathogenic protein. Because cell surface proteins can be utilised as vaccine targets, they are of tremendous interest. PSORTb V3.0 is an algorithm that assigns a protein's likely localization site based on its amino acid sequence.

Prediction of B-cell determinant in proteins: By using a Random Forest algorithm based on epitopes and non-epitope amino acids perceived from crystal structures, the BepiPred-2.0 server predicts B-cell antigenic determinant from a protein sequence. Residues with scores greater than 0.5 are projected to constitute epitopes and are highlighted in yellow on the graph.

RESULTS:

The protein interaction between Fusobacterium nucleatum and triclosan was established using the STITCH pipeline. (Figure 1) By using VirulentPred and VICMpred, the pathogenic properties of proteins interacting with the medicines were evaluated. These algorithms generated ratings that confirmed the protein composition and divided them into two classes: virulent and avirulent.

Triclosan was discovered to react with proteins involved in both cellular and metabolic functions. Possible transcriptional regulator protein associated with information and storage was found to be virulent and its VirulentPred score was 0.7976. Transcriptional regulator, possible transcriptional regulator, possible short chain dehydrogenase, TetR family transcriptional regulator and ADP-glycermanno-heptose 6-epimerase are among the proteins identified as virulence factors and there VirulentPred score was found to be 1.0828, 0.9940, 0.9005, 0.8046, 1.0673 respectively. On the other

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hand, Nitroreductase protein was found to be avirulent. (Table 1) In the pathogenic proteins, the number of anticipated epitopes or antibody binding sites was also determined. (Figure 2)

Figure 1: Network interaction of triclosan with proteins of Fusobacterium nucleatum

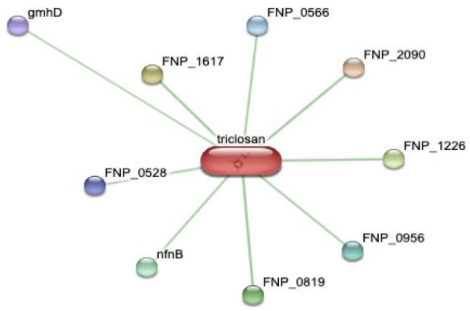
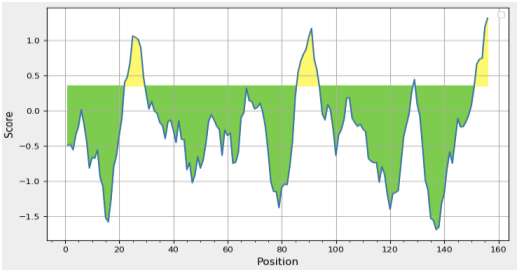
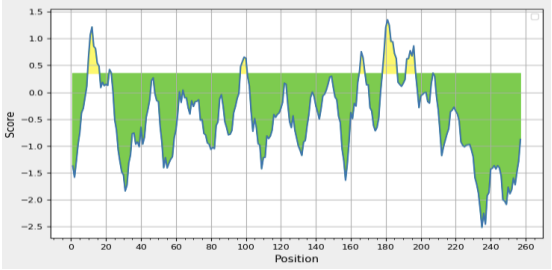


Figure 2: Predicted epitopes on the virulent proteins (a) Transcriptional regulator [FNP_2090], (b) Possible transcriptional regulator [FNP_1226], (c) Possible short-chain dehydrogenase [FNP_0956], (d) TetR family transcriptional regulator [FNP_0819], (e) Possible transcriptional regulator [FNP_1617] and (f) ADP-glyceromanno-heptose 6-epimerase [FNP_1119]



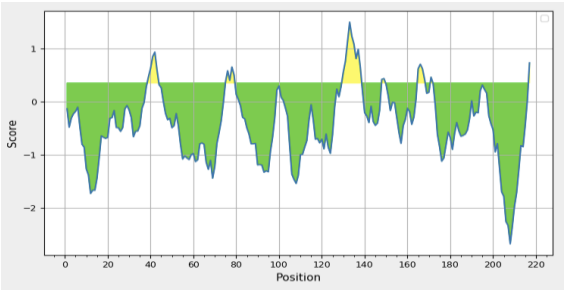
Predicted peptides:

No.	Start	End	Peptide	Length
1	22	29	KEGSDAIT	8
2	86	93	YDTDEEFS	8
3	129	129	N	1



Predicted peptides:

No.	Start	End	Peptide	Length
1	39	43	KAGYN	5
2	75	79	DEISS	5
3	130	138	DDITGQNDI	9
4	148	149	IH	2
5	165	168	ILEE	4
6	171	171	P	1



Predicted peptides:

No.	Start	End	Peptide	Length
1	25	28	IESI	4
2	38	46	DASPAPIYK	9
3	105	106	NI	2
4	122	124	IKK	3
5	133	136	KEKQ	4
6	159	166	FKDPNDSF	8
7	171	174	LRDA	4

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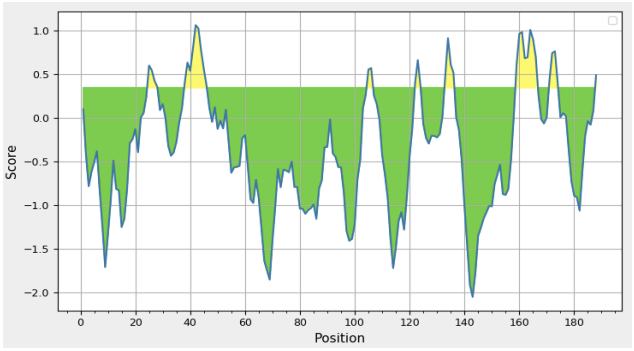
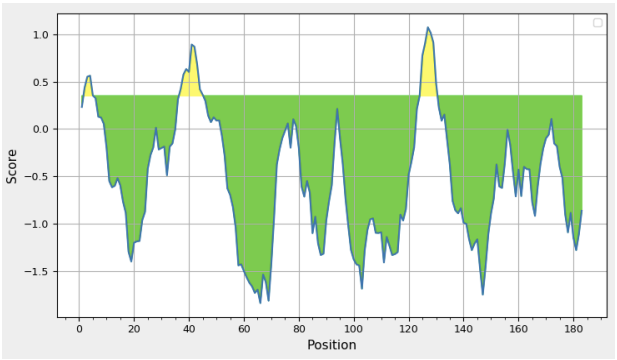


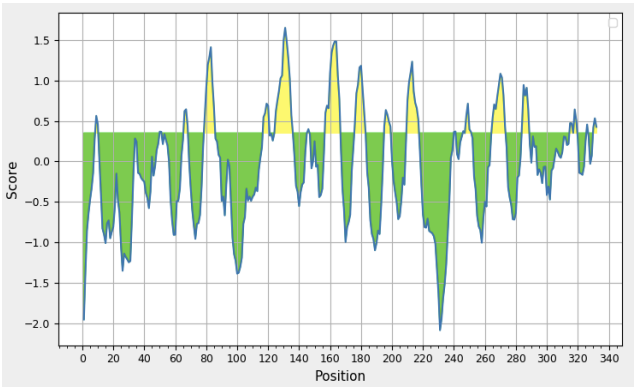
Predicted peptides:

No.	Start	End	Peptide	Length
1	10	16	SSGIGEE	7
2	22	23	AN	2
3	97	100	KEDL	4
4	165	168	LSHK	4
5	179	186	PGPTASNF	8
6	192	197	QEKFGS	6
7	207	207	D	1



Predicted peptides:

No.	Start	End	Peptide	Length
1	2	5	RRKN	4
2	37	45	TSKAGVNRS	9
3	125	130	NNKLTD	6



Predicted peptides:

No.	Start	End	Peptide	Length
1	9	10	GM	2
2	50	51	WI	2
3	66	68	ADK	3
4	79	85	SATTETD	7
5	117	120	AATY	4
6	125	135	LGYNDDVSPEE	11
7	146	146	G	1
8	157	166	FKQKNQPKQW	10
9	176	182	GPQEYHK	7
10	195	199	QYKEN	5
11	210	217	EGFKDGEQ	8
12	240	241	KS	2
13	246	246	I	1
14	248	251	TGKA	4
15	265	273	ASHNDNLDK	9
16	284	288	EDLQG	5
17	315	319	EGVKD	5
18	326	326	A	1

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Table 1: Proteins of Fusobacterium nucleatum interacting with triclosan

Organism	**Identifier**	**Proteins which interacts with triclosan**	**VICMPred Functional Class**	**VirulentPred**	**VirulentPred Score**
Fusobacterium nucleatum	FNP_0566	Nitroreductase	Metabolism Molecule	Non-Virulent	-0.998
	FNP_2090	Transcriptional regulator	Metabolism Molecule	Virulent	1.0828
	FNP_1226	Possible transcriptional regulator	Cellular process	Virulent	0.9940
	FNP_0956	Possible short-chain dehydrogenase	Metabolism Molecule	Virulent	0.9005
	FNP_0819	TetR family transcriptional regulator	Metabolism Molecule	Virulent	0.8046
	FNP_0528	Nitroreductase	Cellular process	Non-Virulent	-0.709
	FNP_1617	Possible transcriptional regulator	Information and storage	Virulent	0.7976
	FNP_0996, nfnB	Nitroreductase	Metabolism Molecule	Non-Virulent	-1.035
	FNP_1119, gmhD	ADP-glyceromanno-heptose 6-eipmerase	Cellular process	Virulent	1.0673

DISCUSSION:

Insilico approaches have become a critical part of the drug development process in recent years. This is due to the fact that they have the ability to alter the entire drug development process, finding and discovering new potential medications while also reducing the requirement for animal models and human cohorts, reducing study time and cost.(10,11) The goal of this research was to find potential interactions between Fusobacterium nucleatum's virulence factor and Triclosan. Periodontal disease, tooth pulp infection, oral cancer, systemic disease, and other infections are all induced by Fusobacterium nucleatum, a common oral bacterium.(12) Triclosan, on the other hand, is an

antimicrobial agent found in a wide range of personal care products, including deodorant soaps, shower gels, and handwashes.(13)

Ushanthika T et al. investigated the identification of virulence factors in reserpine-resistant red complex pathogens. Reserpine can be utilised as an antibacterial agent to remove dental pathogens that are resistant to therapy, according to this study. Reserpine's method of action as an ABC inhibitor will make it an ideal medication for therapeutic use.(14) In vitro and insilico analysis of L.donovani enoyl acyl carrier protein reductase (LdENR) revealed that triclosan persistently interacted with LdENR in the presence of both cofactors (NADPH and NADH), implying that the reduction of L. donovani growth in in vitro and ex vivo drug testing could be attributable to triclosan-LdENR interaction. This research suggests that LdENR could be a therapeutic target for Leishmaniasis, and that triclosan could be a lead.(15)

Target discovery in Fusobacterium nucleatum using subtractive genomics technique and enrichment analysis of host-pathogen protein-protein interactions showed that F. nucleatum infection can expedite CRC progression by concurrently modulating many signal transduction, potentially leading to activation of pro-inflammatory responses, oncogenes, manipulation of the host immune system and suppression of the DNA repair system.(16)

According to a study by Sethi KS et al., both triclosan- and chlorhexidine-coated sutures showed antibacterial properties against periodontal infections.(17) Also, when the distribution of triclosan-resistant genes in key pathogenic bacteria were investigated using metagenome and genome-wide analyses, most human pathogenic bacteria had triclosan resistance determinants.(18)

F. nucleatum was shown to be significant activator of IL-8 production.(19) And Fusobacterium nucleatum is the largest bacteria to infiltrate a mammalian cell, with a length ten times that of Escherichia coli.(20)

The current work identifies multiple important protein interactions of triclosan against F. nucleatum. However, in vitro investigations are needed to investigate the mechanisms that contribute to bacterial susceptibility in order to expand the use of such medications and justify their inclusion in the catalogue of prophylactic agents. Further study in this area could help uncover the correlative and antagonistic effects of these medications when used in amalgamation with standard antibiotics, perhaps opening up new ways to combat lethal infections in the antibiotic-resistant era.

There are some limitations that must be addressed: (a) the interactions driven by the compound towards proteins may be functional or just a physical interaction; (b) homology between the host and pathogen proteins must be validated to avoid any unwanted side effects; and (c) the drug protein interactions recorded by in silico methods may not replicate in a biological environment. As a result, careful experimental designs must be built and tested utilising in vitro and in vivo models.

CONCLUSION:

The current work identifies multiple important protein interactions of triclosan against F. nucleatum. However, a thorough understanding of the interactions between these medications and their antibacterial activity would add to the long list of advantages these treatments have in therapeutic situations. More in vitro research on a huge range of contagious microbes is needed to confirm the genuine interrelation between the medicines and pathogen protein repertoires.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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