Journal of Pharmaceutical Research International

33(47B): 677-683, 2021; Article no.JPRI.74465
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Virtual Screening to Identify the Protein Network Interactions of Triclosan with *Streptococcus mutans* and *Enterococcus faecalis*

T. K. Hariprasanth[1*], J. Vijayashree Priyadharsini[2], A. S. Smiline Girija[2] and P. Sankar Ganesh[2]

[1]Department of Microbiology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, Tamil Nadu, India.
[2]Clinical Genetics Lab, Cellular and Molecular Research Centre, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i47B33169
Editor(s):
(1) Dr. Sawadogo Wamtinga Richard, Ministry of Higher Education, Scientific Research and Innovation, Burkina Faso.
Reviewers:
(1) Mehdi Fakour, Young Researchers and Elite Club, Iran.
(2) Asha Ram Yadav, ICMR- National JALMA institute for Leprosy & Other Mycobacterial Disease, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74465

Original Research Article

Received 04 August 2021
Accepted 09 October 2021
Published 04 November 2021

ABSTRACT

Introduction: Triclosan is considered to be an important ingredient in toothpastes and mouth rinses. Several studies have reported contradictory results regarding the antimicrobial effect of triclosan. Hence, the present in silico study intends to identify the potential targets of triclosan in two common dental pathogens *Streptococcus mutans* and *Enterococcus faecalis.*

Aim: To identify the protein network interactions of triclosan in *Streptococcus mutans* and *Enterococcus faecalis* by virtual screening method.

Materials and Methods: The STITCH v5.0 database was initially used for identifying drug-protein interactions followed by VICMPred and VirulentPred which was employed to identify functional class of the proteins and its virulence property. Finally, BepiPred v1.0 Linear Epitope Prediction tool was used to identify the potential epitopes of the virulent proteins.

Results: Triclosan was found to interact with crucial proteins in *S. mutans* and *E. faecalis* which could contribute to severe forms of periodontitis and endodontic diseases.

**Corresponding author: E-mail: vijayashreej.sdc@saveetha.com;*

Conclusion: Taken together, the present study provides the preliminary data on the potential targets of triclosan in common dental pathogens. Further experimental validation is warranted to provide concrete evidence on the molecular targets of dental pathogens.

Keywords: Triclosan; Streptococcus mutans; Enterococcus faecalis; Periodontitis; antimicrobial agent; novel compounds.

1. INTRODUCTION

Triclosan (TCS), an antimicrobial agent, is present in products such as toothpaste, soaps, detergents, toys, and surgical cleansing solutions [1]. It is a common ingredient in soaps, shampoos, deodorants, toothpastes, mouthwashes, cleaning supplies, and pesticides, and part of consumer products like kitchen utensils, toys, bedding, socks, and trash bags etc [2]. Antimicrobial ingredients have long been used in various products to slow down or inhibit the growth of bacteria, fungi [3]. In hospital settings, 2% triclosan has been used in surgical units for the decolonization of a patient's skin carrying methicillin-resistant *Staphylococcus aureus* (MRSA)[4,5]. A study using commercially available toothpaste containing triclosan indicated a significant reduction in gingivitis, bleeding, and plaque[6] other systematic analysis by Cochrane group suggested that reduction in gingivitis, bleeding, and plaque may be statistically significant under in vitro conditions, but the same could not be replicated in a clinical setting, in other words, triclosan might not attain clinical significance.Our team has extensive knowledge and research experience that has translate into high quality publications[7–11].

Such contradictory results have posed queries about the use of triclosan as an effective anti-microbial agent. Hence, the present study was intended to identify the potential molecular targets of triclosan in common dental pathogens such as *S.mutans* and *E.faecalis.* Computational techniques are widely employed to screen for drug molecules and their targets in microbes and host. Several studies have been initiated by the authors previously to deduce the potential targets of synthetic [12] and phytocompounds [13], [14] against red complexes and other common dental pathogens. Virtual screening methods have been used to reduce cost and time. The prediction tools would provide preliminary information about the molecular targets of the drug in the microbial pathogens, which will be of great use to the researchers to identify drug molecules that would best suit their needs and be less toxic to the hosts.

2. MATERIALS AND METHODS

The present observational study aims to screen for those proteins of *Streptococcus mutans* and *Enterococcus faecalis* interacting with Triclosan. The protein-drug interaction of bacteria was analyzed using STITCH v.5 pipelines [15] and the functional class and virulence property of the interacting proteins was detected using VICMPred and VirulentPred softwares.

2.1 Prediction of Drug-Protein Interactions

The STITCH database helps in providing an exhaustive platform for known and predicted interactions between chemicals and proteins. The interactions could be direct or physical and indirect or functional associations.

2.2 Identification of Virulent Protein and Functional Class

VICMpred [16] and VirulentPred [17] software were used for the identification of virulence factors targeted by Triclosan among the *Streptococcus mutans and Enterococcus faecalis*. 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 VirulentPred tool which classified them into two groups, that is, virulent and avirulent. VICMpred groups proteins into four major classes, namely, proteins involved in cellular processes, metabolism, information storage, and virulence.

2.3 Prediction of Epitopes

The BepiPred v1.0 Linear Epitope Prediction tool predicts B-cell epitopes from a protein sequence, using a Random Forest regression algorithm with trained five fold cross-validation approach was used to discriminate between epitopes and non-epitope amino acid residues 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 [18, 19].

Table 1. Proteins of *Streptococcus mutans* and *Enterococcus faecalis* interacting with triclosan

Organism	Identifier	Proteins which interacts with triclosan	VICMPred Functional Class	Virulent Pred	Virulent Pred Score
Streptococcus mutans	SMU_38c	Transcriptional regulator	Information and Storage	Virulent	0.7941
	SMU_236c	Transcriptional regulator	Metabolism	Virulent	1.0358
	SMU_514	Transcriptional regulator	Cellular process	Virulent	0.7518
	SMU_346	NADH dehydrogenase	Cellular process	Non-virulent	-1.040
	SMU_1602	NAD(P)H-flavin oxidoreductase	Cellular process	Non-virulent	-1.032
	SMU_439	Transcriptional regulator	Cellular process	Virulent	1.0136
	SMU_2134	Transcriptional regulator	Cellular process	Virulent	1.0232
	SMU_1240c	Nitroreductase	Cellular process	Non-virulent	-1.043
	SMU_1343c	Polyketide synthase	Cellular process	Virulent	0.8437
	SMU_1336	Conserved hypothetical protein PksD	Virulence factors	Virulent	1.0032
Enterococcus faecalis	EF_1773	3-ketoacyl-ACP reductase	Cellular process	Virulent	0.7671
	EF_1690	Short chain dehydrogenase/reductase family oxidoreductase	Cellular process	Non-Virulent	.220
	EF_0404	Nitroreductase	Metabolism Molecule	Non-Virulent	-0.998
	EF_3059	TetR family transcriptional regulator	Metabolism Molecule	Virulent	0.8280
	EF_2203	TetR family transcriptional regulator	Cellular Process	Non-Virulent	-1.051
	EF_1181	Nitroreductase	Metabolism Molecule	Non-Virulent	-0.613
	EF_0648	Nitroreductase	Metabolism Molecule	Non-Virulent	-0.990
	EF_1326	TetR family transcriptional regulator	Metabolism Molecule	Virulent	0.8759
	EF_2171	Epimerase/dehydratase	Metabolism Molecule	Virulent	1.0538
	EF_0282	Enoyl-ACP reductase	Metabolism Molecule	Virulent	0.6870

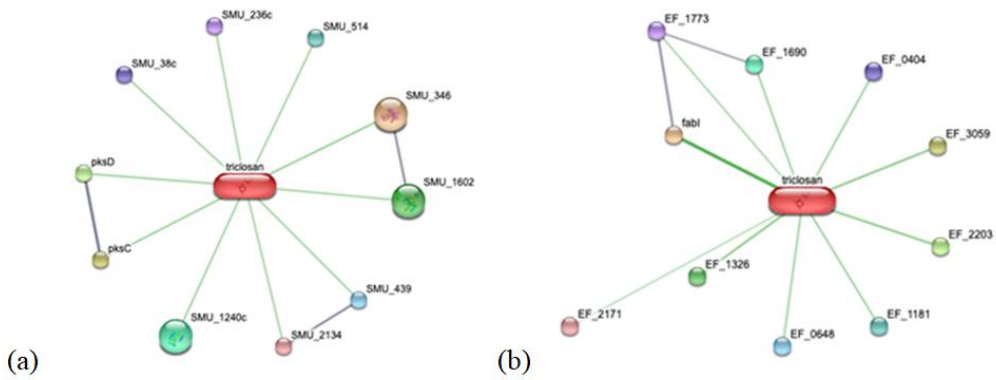

Fig. 1. Protein interaction network of (a) *Streptococcus mutans* and *(b) Enterococcus faecalis*

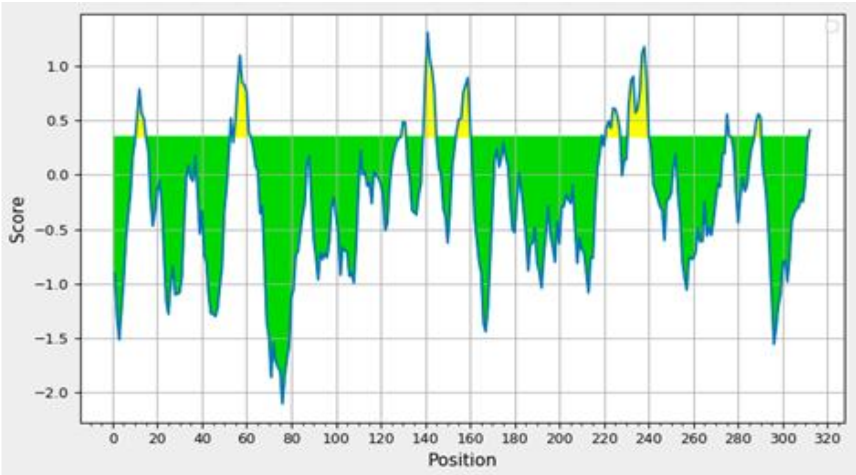

Fig. 2. *Predicted epitopes* on virulent factor conserved hypothetical protein PksD [SMU_1336] identified using computational tools

Predicted peptides:

No.	Start	End	Peptide	Length
1	11	14	GSQY	4
2	53	53	D	1
3	55	61	SKKINTP	7
4	130	131	TN	2
5	139	144	NKDPEQ	6
6	154	160	FAGKFSP	7
7	219	219	Y	1
8	221	227	SSEYEIY	7
9	231	239	RLKTDNNFS	9
10	275	276	TL	2
11	288	290	STS	3

Chart 1. The list of predicted peptide epitopes on the virulent proteins identified

3. RESULTS AND DISCUSSION

Stitch software was used to classify protein interactions between *Streptococcus mutans* and *Enterococcus faecalis* against triclosan (Fig. 1). Furthermore, each of the proteins interacting with the drug was tested for their virulence properties using VirulentPred and functional properties via VICMpred. The scores provided by these algorithms were verified based on their amino acid sequences and patterns which were divided into two groups. i.e., virulent and avirulent. Triclosan was found to interact with crucial proteins in *S. mutans* and *E. faecalis.* Triclosan interacts with more than ten important proteins in both the pathogen, as shown by the virulence properties of 8 virulent proteins in *S. mutans* and 5 virulent proteins in *E. faecalis* in the VirulentPred findings. Most of the virulent proteins identified were transcriptional regulators in *S. mutans* and proteins involved in cellular processes in case of *E. faecalis* (Table 1; Fig 1). Several epitopes were identified in the virulent protein, conserved hypothetical protein PksD of *Streptococcus mutans* (Figs 2 and Chart 1).

Target identification is an important component for the development of therapeutic and diagnostic markers for metabolic, infectious [20-25], autoimmune [26,27,28] and systemic disorders. Computational tools have long been used for the purpose of rapid identification of these potential targets from several sources [29-30]. Dental caries being a serious illness in children has to be identified at the right time and treated using drugs that do not give rise to resistant forms [31-34]. Hence, proper identification of the potential targets has become the need of the hour. We investigated the molecular targets of triclosan and their associations with pathogens in this research, which shows that there are interactions with microbes and virulence factors associated with its functions. Triclosan has been integrated with a number of other dental materials to improve inhibitory effects on microbial metabolism in plaque, calculus, and gingivitis accumulation. As triclosan is used at low concentrations, it inhibits microorganism development; however, when used at higher concentrations, it can disrupt the growth of microorganisms. While the *in silico* methods used provide preliminary evidence on the underlying molecular interaction between the compound and protein network of dental pathogens such as *S. mutans* and *E. faecalis.* The analysis has some limitations, such as (a) the bonding between the compound and the pathogen's protein may be purely physical and (b) the proteins of the red-complex bacteria attacked by the compound may resemble host proteins. To prevent undesirable associations of triclosan with host proteins, in vitro and in vivo studies must be performed to obtain clarification on the healthy use of chemical-compounds on human hosts.

4. CONCLUSION

This study identified molecular targets of triclosan on *S. mutans* and *E. faecalis* which have to be further validated to confirm the critical pathway triggered by the drugs in the physiological conditions. To the best of our knowledge, this study is the first of its kind which aims in understanding the molecular targets of the pathogens against specific drug compounds. The dosage of the drug, minimum inhibitory concentration, and minimum bactericidal concentration against specific microbes should be ascertained by *in vitro* and *in vivo* studies.

FUNDING

We thank Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai for funding the project.

CONSENT

Not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

ACKNOWLEDGEMENT

The authors are thankful to Saveetha Dental college for providing a platform to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jones RD, Jampani HB, Newman JL, Lee AS. Triclosan: A review of effectiveness and safety in health care settings [Internet]. Vol. 28, American Journal of Infection Control. 2000;184–96.

Available:http://dx.doi.org/10.1067/mic.2000.102378

2. Dann AB, Hontela A. Triclosan: environmental exposure, toxicity and mechanisms of action [Internet]. Vol. 31, Journal of Applied Toxicology. 2011;285–311.
Available:http://dx.doi.org/10.1002/jat.1660
3. McAvoy DC, Schatowitz B, Jacob M, Hauk A, Eckhoff WS. Measurement of triclosan in wastewater treatment systems [Internet]. Vol. 21, Environmental Toxicology and Chemistry. 2002;1323–9.
Available:http://dx.doi.org/10.1002/etc.5620210701
4. Suller MTE. Triclosan and antibiotic resistance in Staphylococcus aureus [Internet]. Vol. 46, Journal of Antimicrobial Chemotherapy. 2000;11–8.
Available:http://dx.doi.org/10.1093/jac/46.1.11
5. Villalaín J, Reyes Mateo C, Aranda FJ, Shapiro S, Micol V. Membranotropic effects of the antibacterial agent triclosan [Internet]. Vol. 390, Archives of Biochemistry and Biophysics. 2001;128–36.
Available:http://dx.doi.org/10.1006/abbi.2001.2356
6. Schweizer HP. Triclosan: A widely used biocide and its link to antibiotics [Internet]. Vol. 202, FEMS Microbiology Letters. 2001;1–7.
Available:http://dx.doi.org/10.1111/j.1574-6968.2001.tb10772.x
7. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. An insight into the emergence of *Acinetobacter baumannii* as an oro-dental pathogen and its drug resistance gene profile – An in silico approach [Internet]. Vol. 4, Heliyon. 2018;e01051.
Available:http://dx.doi.org/10.1016/j.heliyon.2018.e01051
8. Priyadharsini JV. *In silico* validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens [Internet]. Vol. 90, Journal of Periodontology. 2019;1441–8.
Available:http://dx.doi.org/10.1002/jper.18-0673
9. Balamithra S, Girija S, Vijayashree Priyadharsini J. An *in silico* analysis of protein targeted by glycyrrhizin in common dental pathogens [Internet]. Journal of Pharmaceutical Research International. 2020;170–8.
Available:http://dx.doi.org/10.9734/jpri/2020/v32i1530640
10. Nandhini JST, Thaslima Nandhini JS, Smiline Girija AS, Vijayashree Priyadharsini J. Virtual screening to identify the protein targets in common dental pathogens interacting with menthol [Internet]. Journal of Pharmaceutical Research International. 2020;25–31.
Available:http://dx.doi.org/10.9734/jpri/2020/v32i2130749
11. Nivethitha R, Smiline Girija AS, Vijayashree Priyadharsini J. An observational study on the mode of action of ferulic acid on common dental pathogens – an in silico approach [Internet]. Journal of Pharmaceutical Research International. 2020;13–20.
Available:http://dx.doi.org/10.9734/jpri/2020/v32i1830684.
12. Ushanthika T, Smiline Girija AS, Paramasivam A, Priyadharsini JV. An *in silico* approach towards identification of virulence factors in red complex pathogens targeted by reserpine. Nat Prod Res. 2021; 35(11):1893–8.
13. Ramalingam AK, Selvi SGA, Jayaseelan VP. Targeting prolyl tripeptidyl peptidase from Porphyromonas gingivalis with the bioactive compounds from Rosmarinus officinalis. Asian Biomed. 2019;13(5):197–203.
14. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with *Murraya koengii* bio-compounds: An *in-silico* approach. Acta Virol. 2020;64(1):93–9.
15. Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Kuhn M. STITCH 5: augmenting protein-chemical interaction networks with tissue and affinity data. Nucleic Acids Res. 2016;44(D1):D380–4.
16. Saha S, Raghava GP. VICMpred: an SVM-based method for the prediction of functional proteins of Gram-negative bacteria using amino acid patterns and composition. Genomics Proteomics Bioinformatics. 2006;4(1):42-7.
DOI: 10.1016/S1672-0229(06)60015-6.
17. Garg A, Gupta D. VirulentPred: a SVM based prediction method for virulent proteins in bacterial pathogens. BMC Bioinformatics. 2008 28;9:62.
18. Jespersen MC, Peters B, Nielsen M, Marcatili P. BepiPred-2.0: improving sequence-based B-cell epitope prediction

using conformational epitopes [Internet]. 45, Nucleic Acids Research. 2017;W24–9. Available:http://dx.doi.org/10.1093/nar/gkx346.

19. Larsen J, Lund O, Nielsen M. Immunome Research [Internet]. Vol. 2. 2006;2. Available:http://dx.doi.org/10.1186/1745-7580-2-2
20. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. Hypertens Res. 2020;43(12): 1459–61.
21. Paramasivam A, Vijayashree Priyadharsini J, Raghunandhakumar S. N6-adenosine methylation (m6A): A promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res. 2020;43(2):153–4.
22. Jayaseelan VP, Arumugam P. Exosomal microRNAs as a promising theragnostic tool for essential hypertension. Hypertens Res. 2020;43(1):74–5.
23. Girija AS. Fox3 (+) CD25 (+) CD4 (+) T-regulatory cells may transform the nCoV's final destiny to CNS! COMMENT. WILEY 111 RIVER ST, HOBOKEN 07030-5774, NJ USA; 2021.
24. Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-Induced hyperinflammation magnify the severity of coronavirus disease (CoViD-19) leading to acute respiratory distress syndrome? Front Immunol. 2020;11:1206.
25. Iswarya Jaisankar A, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterisation of csgA gene among ESBL strains of *A. baumannii* and targeting with essential oil compounds from *Azadirachta indica*. Journal of King Saud University - Science. 2020;32(8): 3380–7.
26. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent. 2021;31(3):436–41.
27. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species [Internet]. Vol. 94, Archives of Oral Biology. 2018;93–8. Available:http://dx.doi.org/10.1016/j.archoralbio.2018.07.001.
28. Paramasivam A, Vijayashree Priyadharsini J. Novel insights into m6A modification in circular RNA and implications for immunity. Cell Mol Immunol. 2020;17(6):668–9.
29. Paramasivam A, Priyadharsini JV, Raghunandhakumar S. Implications of m6A modification in autoimmune disorders. Cell Mol Immunol. 2020;17(5): 550–1.
30. Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from *Ganoderma lucidum*: A computational study. pharmaceutical-sciences [Internet]. 2020; 82(2). Available:https://www.ijpsonline.com/articles/targeting-nm23h1mediated-inhibition-of-tumour-metastasis-in-viral-hepatitis-with-bioactive-compounds-from-ganoderma-lucidum-a-comp-3883.html.
31. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021;31(2):285–6.
32. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021; 126:105132.
33. Teja KV, Ramesh S. Is a filled lateral canal - A sign of superiority? J Dent Sci. 2020; 15(4):562–3.
34. Reddy P, Krithikadatta J, Srinivasan V, Raghu S, Velumurugan N. Dental caries profile and associated risk factors among adolescent school children in an urban south-Indian city. Oral Health Prev Dent. 2020;18(1):379–86.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74465