Virtual Screening to Identify the Protein Network Interaction of Triclosan in Red Complex Pathogens

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Authors’ contributions

This work was carried out in collaboration among all authors. Author NVHR carried out the literature search, data collection, data analysis and manuscript writing. Author JVP has conceived the study, participated in its design and coordinated and provided guidance to draft the manuscript. Authors PSG and ASSG equally contributed in the validation and development of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Antimicrobial drug resistance is the major problem encountered world-wide. Novel therapeutic leads have been identified and are regularly tested for their activity against microbial pathogens.

Aim: To identify the protein network interactions of triclosan in red complex pathogens.

Materials and Methods: The present study follows an observational study design which aims to screen for the interaction of triclosan in red complex pathogens. The interaction was analyzed using the STITCH v.5 pipeline. The functional class of proteins identified were assessed using VICMPred and VirulentPred softwares. The microbial pathogens Treponema denticola ATCC 35405, Tannerella forsythia ATCC 43037, Porphyromonas gingivalis ATCC 33277 are the strains of red complex pathogens that are included in the present study.

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Results and Discussion: Several proteins were found to interact with triclosan. Among the protein interactions, interactions of triclosan with virulent proteins seems to have a greater impact. The NAD-dependent nucleotide-diphosphate-sugar epimerase [PGN_1370], Putative NAD dependent epimerase [PGN_1886], GDP-fucose synthetase [PGN_1079], Probable oxidoreductase [PGN_1360] of Porphyromonas gingivalis, Conserved hypothetical protein [TDE_2401], Epimerase/dehydratase family protein [TDE_1439] of Treponema denticola, NAD dependent epimerase/dehydratase family protein [BFO_2919], Hypothetical protein [BFO_1782], Nitroreductase family protein [BFO_1604] and Nitroreductase family protein [BFO_1516] Tannerella forsythia were found to be exhibit virulence nature.

Conclusion: This study identifies the molecular targets of triclosan on red complex pathogens. As triclosan interacts with the red complex pathogens, in future it can be used as a primary medicine for periodontitis and some oral conditions.

Keywords: Triclosan; protein network interaction; red complex pathogens; periodontitis; novel targets.

1. INTRODUCTION

Periodontitis is the most prevalent problem encountered in the dental settings. Periodontitis can be classified as adult periodontitis, rapidly progressive periodontitis and refractory adult periodontitis [1]. The organisms most commonly associated with periodontitis are Actinobacillus actinomycetemcomitans, Bacteroides forsythus, Pre-votella intermedia, Streptococcus intermedia, Eubacterium nodatum, etc. Apart from these organisms the red complex pathogens seems to be the major culprits behind periodontitis. Protein network interactions are statistical presentations of contact between proteins in a cell and the drug [2,3]. Protein network interaction will represent both the stable and transient interactions [3].

Triclosan was first reported to be effective against bacteria and fungi. Mouthwash, bar soap, liquid soap, shower gels, face washes/cleansers, hair shampoos, underarm deodorants, shaving creams, after-shave lotion and anti-acne preparations all now contain triclosan [4]. It has been a widely approved broad spectrum antimicrobial agent which is successful in opposition to many gram negative and gram positive bacteria [5]. The anti gingivitis and antiplaque effectiveness of triclosan in containing debris is accepted. Our team has extensive knowledge and research experience that has translate into high quality publications [6–10].

The emergence of a newer drug resistant community has largely hampered the process of therapy [11,7,8,12]. It is useful as antiseptics to destroy the bacteria on the surfaces of skin and also used in medical devices to prevent the products from microbial decay [9]. It prevents bacterial fatty acid synthesis. Fab pathway is a main target for antimicrobial agents [13]. Several synthetic and phytocompounds have been assessed using in silico methods to identify potential protein targets in common dental pathogens [14,15]. The present study is to identify the protein network interaction of triclosan in red complex pathogens

2. MATERIALS AND METHODS

2.1 Study Design

The present study follows an observational study design which aims to screen for the interaction of triclosan in red complex pathogens. The interaction was analysed using STITCH v.5 pipeline [16]. The functional class of proteins identified were assessed using VICMPred [17] and VirulentPred softwares [18]. The microbial pathogens Treponema denticola ATCC 35405, Tannerella forsythia ATCC 43037, Porphyromonas gingivalis ATCC 33277 are the strains of red complex pathogens that are included in the present study.

2.2 Prediction of Protein-drug Interactions

To predict the interactions between proteins and chemicals STITCH database (Version 5; 2016) is used. The interactions include associations of direct or physical and indirect or functional is used for the computational prediction and from the responses the data is aggregated. The repertoire of proteins which interacts with T. forsythia, P. gingivalis and T. denticola and were further used for predicting virulence [16].

2.3 Virulence Prediction

For the identification of virulence factors the software used was VICM pred [17] and Virulent
Pred [18] pipelines. VICMPred groups proteins are classified into four major classes: proteins involved in metabolism, information storage, virulence and cellular processes. The overall accuracy of VirulentPred servers and VICMPred were 86% and 70.75%, respectively.

2.4 Prediction of Subcellular Localization of the Virulent Proteins

The novel drug targets plays an important role in an antimicrobial drug which targets the virulent protein. The subcellular localization of proteins aids in designing using the Computational prediction. The great interest is that cell surface proteins can be used in making vaccines. An algorithm which assigns a probable localization site to a protein from an amino acid sequence is pSORTb V3.0 [19].

2.5 Prediction of B-cell Epitopes in the Virulent Proteins

For the prediction of B-cell epitopes from a protein sequence the server is BepiPred-2.0 was used. To be part of an epitope the residues with scores above the threshold (>0.5) [20,21].

3. RESULTS AND DISCUSSION

Pathogens of the red complex, such as P. gingivalis, T. denticola, and T. forsythia, are important contributors to periodontal infections. The removal of these pathogens from the infection site is still a challenge. In silico tools have been largely used to cut down the primary cost of screening active molecules for their antimicrobial effect [22]. Gomez, et al investigated the inhibitory and lethal effect of triclosan against several microorganisms at different stages of their phase of population growth. Several proteins were found to interact with triclosan [20]. Among the protein interactions, interactions of triclosan with virulent proteins seems to have a greater impact (Fig. 1). The NAD-dependent nucleotide-diphosphate-sugar epimerase [PGN_1370], putative NAD dependent epimerase [PGN_1886], GDP-fucose synthetase [PGN_1079], probable oxidoreductase [PGN_1360] of Porphyromonas gingivalis, conserved hypothetical protein [TDE_2401], Epimerase/dehydratase family protein [TDE_1439] of Treponema denticola, NAD dependent epimerase/dehydratase family protein [BFO_2919], hypothetical protein [BFO_1782], nitroreductase family protein [BFO_1604] and nitroreductase family protein [BFO_1516] Tannerella forsythia were found to exhibit virulence nature (Table 1). Most of the proteins identified as virulent were located in the cytoplasm (Table 2). Several virulent proteins identified were found to possess multiple epitopes demonstrating a greater tendency to elicit immune response in the host (Fig. 2).

Several in silico studies have been performed to predict the potential targets of microbial pathogens against the drug selected [21-23]. Farsi and Tanner, performed an in vitro study to analyse the resistance of Porphyromonas gingivalis, Prevotella intermedia, and Tannerella forsythia to triclosan. No growth of Porphyromonas gingivalis and P. intermedia were observed in plates containing ≥ 2 μg/ml triclosan, while T. forsythia did not grow on ≥ 1.66 μg/ml. Resistant strains of P. intermedia triclosan developed after prolonged incubation at 2 μg/ml [24]. Our research team has performed several studies related to computational analysis related to infectious diseases, metabolic, autoimmune disorders and cancer [25-26].

Table 1. Proteins of red complex pathogens interacting with triclosan

| Organism          | Identifier | Proteins which interacts with triclosan | VICMPred Functional Class | Virulent Pred | Virulent Pred Score |
|-------------------|------------|----------------------------------------|---------------------------|--------------|---------------------|
| Porphyromonas gingivalis | PGN_1370  | NAD-dependent nucleotide-diphosphate-sugar epimerase | Cellular process           | Virulent     | 0.6274              |
|                   | PGN_0224  | UDP-N-acetyl-D-mannosaminuronic acid dehydrogenase | Cellular process           | Avirulent    | -1.372              |
|                   | PGN_1886  | NAD dependent epimerase                 | Cellular process           | Virulent     | 0.9781              |
|                   | PGN_1079  | GDP-fucose synthetase                   | Metabolism Molecule Cellular process | Virulent     | 1.0619              |
|                   | PGN_0365  | Arginyl-tRNA synthetase                 | Cellular process           | Avirulent    | -1.099              |
|                   | PGN_1652  | Nitroreductase                          |                           | Avirulent    | -1.006              |
Organism | Identifier | Proteins which interacts with triclosan | VICMPred Functional Class | Virulent Pred | Virulent Pred Score
--- | --- | --- | --- | --- | ---
**Treponema denticola** | PGN_0765 | Nitroreductase | process Metabolism Molecule Molecule | Avirulent | -1.053
| PGN_1360 | Oxidoreductase | | | | 1.0723
| TDE_2401 | Hypothetical protein | Metabolism Molecule Molecule Molecule | Virulent | 0.7963
| TDE_0708 | Nitroreductase | | | Avirulent | -0.405
| TDE_1439 | Epimerase/dehydratase | Cellular process | Virulent | 0.9466
| TDE_1363 | Nitroreductase | Metabolism Molecule Molecule Molecule | Avirulent | -1.074
| TDE_1953 | TetR family transcriptional regulator | Metabolism Molecule Molecule Molecule | Avirulent | -1.081
| TDE_0246 | TetR family transcriptional regulator | Metabolism Molecule Molecule Molecule | Avirulent | -0.345
**Tannerella forsythia** | BFO_2919 | NAD dependent epimerase/dehydratase family protein | Cellular process | Virulent | 0.7052
| BFO_1051 | Nucleotide sugar dehydrogenase | Cellular process | Avirulent | -0.113
| BFO_3081 | GDP-L-fucose synthetase | Metabolism Molecule Molecule Molecule | Avirulent | -0.234
| BFO_3174 | Nitroreductase family protein | Cellular process | Avirulent | -1.062
| BFO_1782 | Hypothetical protein | Cellular process | Virulent | 0.7487
| BFO_1604 | Nitroreductase family protein | Metabolism Molecule Molecule Molecule | Virulent | 1.0084
| BFO_1516 | Nitroreductase family protein | Cellular process | Virulent | 1.0676
| BFO_1640 | Arginine--tRNA ligase | Metabolism Molecule Molecule Molecule | Avirulent | -1.055
| BFO_0712 | Nitroreductase family protein | Metabolism Molecule Molecule Molecule | Avirulent | -0.984
| BFO_1683 | Short chain dehydrogenase/reductase family oxidoreductase | Metabolism Molecule Molecule Molecule | Avirulent | -0.924

Table 2. Subcellular location of virulent proteins from red complex pathogens

| Virulent protein | Subcellular location | Score |
|-----------------|---------------------|-------|
| NAD-dependent nucleotide-diphosphate-sugar epimerase | Cytoplasmic | 8.96 |
| Putative NAD dependent epimerase | Cytoplasmic | 8.96 |
| GDP-fucose synthetase | Cytoplasmic | 9.97 |
| Probable oxidoreductase | Unknown | - |
| Epimerase/dehydratase | Cytoplasmic | 8.96 |
| Conserved hypothetical protein | Cytoplasmic | 8.96 |
| NAD dependent epimerase/dehydratase family protein | Cytoplasmic | 8.96 |
| Hypothetical protein BFO_1782 | Cytoplasmic/Membrane | 9.82 |
| Nitroreductase family protein | Unknown | - |
| Nitroreductase family protein | Cytoplasmic | 8.96 |
Fig. 1. Protein interaction network of (a) *Porphyromonas gingivalis* (b) *Treponema denticola* and (c) *Tannerella forsythia* with triclosan.

Fig. 2. Predicted epitopes in virulent proteins (A) NAD-dependent nucleotide-diphosphate-sugar epimerase [PGN_1370], (B) Putative NAD dependent epimerase [PGN_1886], (C) GDP-fucose synthetase [PGN_1079], (D) Probable oxidoreductase [PGN_1360] of *Porphyromonas gingivalis*, (E) Conserved hypothetical protein [TDE_2401], (F) Epimerase/dehydratase family protein [TDE_1439], *Treponema denticola* (G) NAD dependent epimerase/dehydratase family protein [BFO_2919], (H) Hypothetical protein [BFO_1782], (I) Nitroreductase family protein [BFO_1604], (J) Nitroreductase family protein [BFO_1516] *Tannerella forsythia*.
4. LIMITATIONS

The limitations of the present study is that the protein interactions demonstrated here may not work the same way in a complex biological system. Also, sometimes the microbial proteins mimicking host proteins could bring about cross reactions with the bioactive compound.

5. FUTURE SCOPE

Computational methods have supported basic science researchers by cutting down the time required for analysis of numerous bioactive compounds and screening the best suitable compound which works against specific pathogens. The study can be further extended in an in vitro and in vivo set up to provide more evidence on the antimicrobial effect of the compound against dental pathogens.

6. CONCLUSION

This study identifies the molecular targets of triclosan on red complex pathogens. As triclosan interacts with the red complex pathogens, in future it can be used as a primary medicine for periodontitis and some oral conditions.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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