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Computational Analysis of Action of Fatty Acids from Coconut and Monolaurin on Peptide Deformylase of *Clostridium difficile*

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ABSTRACT: Increasing occurrence of pathogen resistance to antibiotics has reached alarming levels that poses a need to identify new classes of drugs. Peptide deformylase (PDF), a metalloenzyme and a promising bacterial target, may prove to be a wise choice since it executes the deformylation of N-formyl group form nascent polypeptide chains during protein synthesis in most pathogens. Therefore, this metalloproteinase is crucial for bacterial survival. Lipid components of Virgin Coconut Oil (VCO) have been known to exhibit a broad spectrum of antibacterial activity. In order to predict their antibacterial mechanisms, we have used an *in silco* modeling and docking approach. We screened the binding affinity of different fatty acid and monolaurin (a metabolite of lauric acid) against PDF enzyme of *Clostridium difficile*, which causes diarrhoea. Lauric acid (C12) and its metabolite, monolaurin displayed considerable binding affinity against PDF of *C. Difficile*, with the interaction patterns revealed the presence of more number of H-bonds with PDF of *C. difficile* active site residues. Similar pattern was observed in other medium chain fatty acids, such as capric (C10) and caprylic (C8) protein models. The binding pattern of these medium chain fatty acids with peptide deformylase protein models may provide insights into design new potent drugs to control emerging antibiotic resistant pathogenic bacterial strains.

KEYWORDS: peptide deformylase (PDF), *Clostridium difficile*, Virgin Coconut Oil (VCO), fatty acids, monolaurin

I. INTRODUCTION

Antibiotic resistance of microorganisms is a consequence of their evolutionary adaptation due to the indiscriminate use of antibiotics. Tremendous adverse effects are found associated with the use of synthetic antibiotics. The emergence of multidrug resistance of pathogen is also a serious threat. This necessitates a continuous need to discover new antimicrobial compounds, possessing novel mechanisms of action against infective diseases. Researchers are now turning their attention to extract and analyse compounds of plant origin to control these pathogens. Previous studies have shown the antimicrobial properties of ethanolic extract of citrus leaves on *Escherichia coli* and *Pseudomonas aeruginosa* [1]. Essential oil of some selected plants, cloves and cinnamon have been analysed and found active against methicillin resistant S*taphylococcus aureus* [2]. Therefore, extraction of new phytocompounds and study of their mode of action would definitely enable development of new antimicrobial drugs, which are cost effective and possessing no adverse effects.

Virgin Coconut Oil (VCO), derived from coconut milk, has been reported to possess a lot of medical potential with its antibacterial, antifungal and anti viral properties exerted by its medium chain fatty acids (MCFAs), mainly lauric acid (C12: 0) and also in its monoglyceride form (monolaurin) [3,4]. It is bound in the form of triglycerides in the VCO and is converted to lauric acid and monoglyceride in the human gastrointestinal tract [5]. In addition, MCFA are easily absorbed into cells and then to mitochondria [5]. Of the free fatty acids present in coconut oil, lauric acid (C12:0) has been reported to more active as an antibacterial compared to caprylic acid (C8:0), capric acid (C10:0) and myristic

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acid (C14:0) [6]. Antibacterial effects of free fatty acids and monoglycerides of VCO have been hypothesized to inactivate bacteria by disrupting plasma membrane of lipid bilayer [6].

In the present study, we have explored the potential of free fatty acids and monolaurin as an antibacterial agent through the inhibition of peptide deformylase (PDF) of *Clostridium difficile* using *in silico* modelling and docking approaches. *C. difficile* is the causative agent of hospital acquired antibiotic associated diarrhoea worldwide, and its proliferation creates a significant problem. PDF is found expressed in all pathogenic bacteria, including *Clostridium*, and is essential for prokaryotic protein maturation; PDF removes formyl group from methionine at the N-terminus of nascent polypeptide chain. There is no functionally equivalent gene in mammalian system. Inhibition of PDF may, therefore, be a target to control pathogenic bacteria and such studies might provide insights for development of novel drugs to inhibit these enzymes.

II. MATERIALS AND METHODS

In the present study, three dimensional structural model of the peptide deformylase protein of *Clostridium difficile* (EQJ11455.1) were generated. Validation of the constructed model was carried out using Ramachandran plots generated in PROCHECK and 3D scores profiles of discovery studio software v 2.0. Docking analyses of PDF protein with fatty acids were performed to identify antimicrobial interactions among them. Different methods used in this study are highlighted below:

Homology modeling: The 3D modeling of PDF protein of *Clostridium difficile* was performed using DS Modeling 2.0. The homologous sequence search and alignment were done with two modules, sequence analysis and protein families. PDF sequences of six other *Clostridium* sp. (*C. innocuum, C. perfringens, C. tetani, C. botulinum, C. thermocellum* and *C.beijerinckii)* were identified by searching the NCBI website. Multiple sequence alignment was generated using sequence and structural information of PDF protein of *Clostridium* spp. and with its templates in ClustalW. The final 3D PDF protein model was generated by MODELER program of DS Modeling studio 2.0, which includes automated homology and loop modeling.

Protein simulation: PDF protein of *C. difficile* models, were further subjected to refinement by CHARMm [7] in DS Modeling 2.0. CHARMm provides powerful mechanical and dynamical protocols for studying the energetic and motion of molecules, from small ligands to multi-components. Throughout protein simulation was done using CHARMm force field, constraint was applied to allow only binding site and ligand to be flexible during the simulation.

Ligand identification and selection: We have selected medium chain fatty acids and monolaurin of VCO for *in silico* prediction of their antimicrobial activity. Compounds were screened from PubChem Compound Database (http://www.ncbi.nlm.nih.gov/pccompound) and the PDB structure of fatty acids and monolaurin were deduced using PRODRG Server (http://davapc1.bioch.dundee.ac.uk/prodrg/).The PubChem ID and structure of each ligand is given in Table 1.

Protein-ligand interaction study: LigandFit/LigandScore, is an automated docking/scoring tool [8] in DS Modeling 2.0, was used for this study, which includes:

Ligand-/cavity based, defined binding site, different ligand confirmation generation, align ligand shapes to binding sit, docking each conformation, top docked structures save in diverse poses, best binding affinity prediction and calculating binding score for each docked structure, ligandfit Score (DS), dock Score (force field) = - (ligand/receptor interaction energy +ligand internal energy).
Different structural models of PDF protein of various *Clostridium* spp. were docked with different VCO derived fatty acids and monolaurin. All the ligand structures were downloaded from PubChem database as *.sdf file. In addition, docking was performed to find the inhibition activity of fatty acids and monolaurin against *C. difficile* PDF using Hex version 6.0 (http://hex.loria.fr/dist60/).

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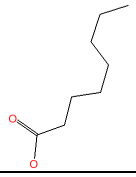
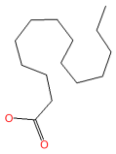
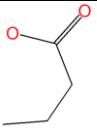
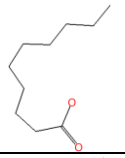
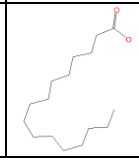
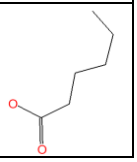
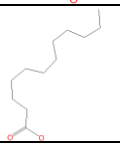
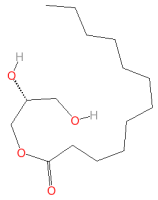
Table 1: Structures of fatty acids from virgin coconut oil and monolaurin

Compound Name	Pubchem ID	Structure	Compound Name	Pubchem ID	Structure	Compound Name	Pubchem ID	Structure
Medium Chain Fatty Acids			**Long Chain Fatty Acids**			**Short Chain Fatty Acids**		
Caprylic Acid	CID379		Myristic Acid	CID11005		Butyric Acid	CID264	
Capric Acid	CID 2969		Palmitic Acid	CID985		Caproic Acid	CID8892	
Lauric Acid	CID3893							
Monoglyceride								
Monolaurin	CID14871							

III. RESULTS AND DISCUSSION

Similarity and structure prediction of Clostridium PDF: From BLAST and multiple sequence alignment analyses, it could be seen that PDF proteins of the six other *Clostridium* spp. shared an identity from 27-99% with *C. difficile* (Fig. 1). *C. innocuum* showed maximum percentage identity with PDF of *C. difficile*, while *C. beijerinckii* of minimum identity. The phylogenetic analysis showed that PDF of *C. innocuum*, *C. difficile* and non- spore forming *Eubacterium*, formed a seperate cluster, from *C. beijerinckii.* In addition, other *Clostridium* spp. formed a distinct cluster in the phylogenetic tree (Fig. 2). Evolution of PDF protein of different *Clostridium* spp. might have occurred in different periods during evolution [9].

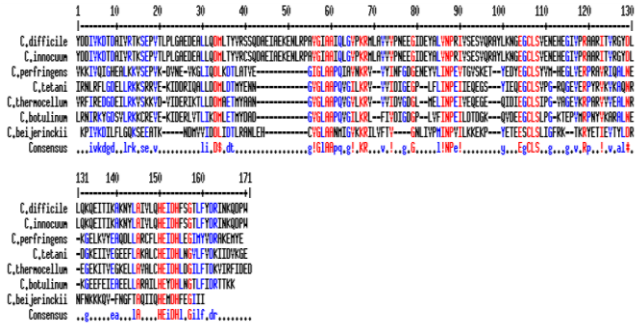


Fig.1. Multiple sequence alignment showing identity among the PDF sequence of *Clostridium* sp.

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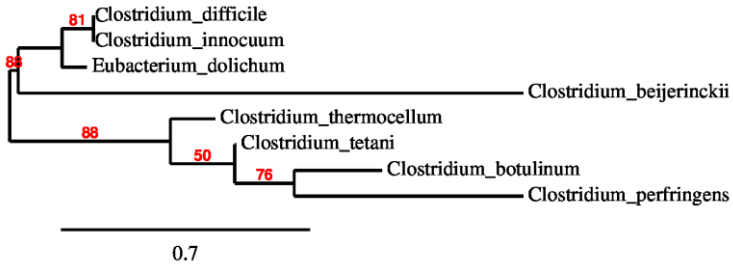

Fig. 2. Phylogenetic tree among different *Clostridium* and *Eubacterium* spp. based on PDF sequences. Numbers at internal nodes are corresponding to bootstrap support values, obtained in the analysis of 100 replicates .

Distant homologues were selected for modeling PDF protein using MODELER program. The PDB ids of selected templates were 2OS0, 1Q1Y and 3CMD. PDB '2OS0' is the crystal structure of actinonin bound peptide deformylases of *Enterococcus faecalis* and *Streptococcus pyogenes*, PDB '1Q1Y' is of *Staphylococcus aureus* and PDB '3CMD' is of VRE-*Enterococcus faecium*. The helix-turn helix structure of PDF protein of *C. difficile* was clearly revealed from the experiment model and visualized in Rasmol (Fig. 3a and Fig. 3b). Amino acids 31-39, 49-54 and 97-102 were responsible for formation of first, second and short third helix respectively. Protein model validation, carried out with Ramachandran plot computed by PROCHEK program, showed 98.7% residues in favored and allowed regions (Fig. 3c). No invalid region was found in the modeled structures of PDF protein. Loop modeling and side chain refinement did not yield any further change in the models.

Ligand-protein interaction: Ligand-protein interaction studies between C. difficile PDF with fatty acids from virgin coconut oil and monolaurin has not yet been reported. Molecular docking studies revealed different ligand binding sites in the PDF protein. Different binding conformations of the ligands with the protein were scored and analysed using LigandFit score. More than 50 docking were performed in DS for finding the optimum ligand protein interactions of various ligands taken for the study.

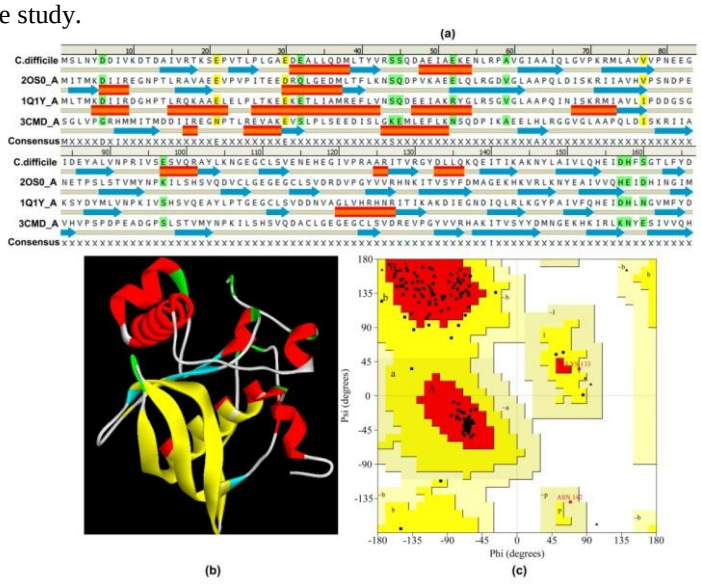

Fig. 3 (a): Alignment of templates and target (PDF protein of *C.difficile*) performed by Discovery Studio 2.0. Predicted secondary structure of PDF protein in which helices are colored red, superimpose in β-strands (blue). (b): A screenshot (from DS) of predicted alpha helical structure of PDF protein of *C.difficile.* (c) Ramachandran plot for the model of PDF protein of *C.difficile* (98.7% amino acid residues of the model are in favored and allowed region).

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The ligand binding sites of PDF modeled structure was predicted in order to define the inhibition receptor function. The four ligand binding sites, site I- X, Y, Z- 38.4, 11.9, 28.6; site II (X, Y, Z- 56.2, 16.9, 20.1); site III (X,Y,Z- 48.4, 26.4, 21.1); site IV (X,Y,Z- 55.2,17.1,33.1) with default parameters of grid spacing 0.5, grid angle 90^0 and threshold value of 2.5 were visible and is shown in Fig. 4.

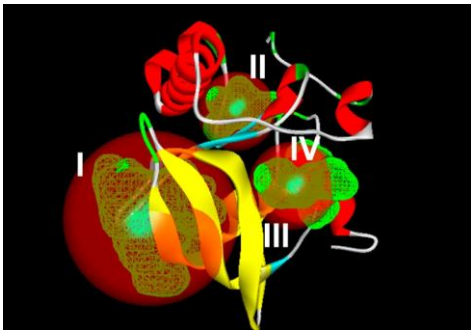

Fig. 4. Four signature binding sites of PDF protein of *C. difficile*

For docking analysis, ligand 3D structure was screened and docked onto the predicted active site of PDF protein model using DS studio visualizer. In addition, once the docking was completed, the generated results gave a corresponding e-value for each docked structure. The more negative the e-value, the more efficient is the docking. Different ligands selected for the study include medium chain fatty acids, long and short chain fatty acids and monolaurin. Independent runs were carried out for each ligand. The minimum binding energies of all eight ligands with protein models are listed in Table 2. A ligand undergoes either hydrophobic or hydrogen bond or both with the active site of the target protein while docking. Docking analysis of fatty acids and monolaurin with PDF protein revealed that monolaurin (CID14871) and medium chain fatty acids such as lauric (CID3893), caprylic (CID379), capric (CID 2969) acids show an effective and very efficient binding with the protein model in the study (Fig.5 a, b, c & d). A dock score above 30 signifies efficient interaction between protein and its ligand. Short chain fatty acids were found to be minimally effective against the selected protein models. Energy values obtained are tabulated in Table 2.

Species Name	Protein	Ligands	Effective docking interaction value (e-value)	Dock Score
Clostridium difficile	Peptide deformylase (PDF)	Lauric Acid	-228.32	78.65
		Monolaurin	-253.95	85.93
		Caprylic Acid	-201.45	68.51
		Capric Acid	-164.04	63.31
		Myristic Acid	-117.32	46.28
		Palmitic Acid	-128.34	52.76
		Butyric Acid	-103.12	20.85
		Caproic Acid	-97.31	28.76

Table 2. Results of docking using Hex Server

When viewed in visualization tool like RASMOL, the effective docking interaction (significant e-value) between receptors of proteins and the ligand could clearly be observed as shown in Fig. 5.

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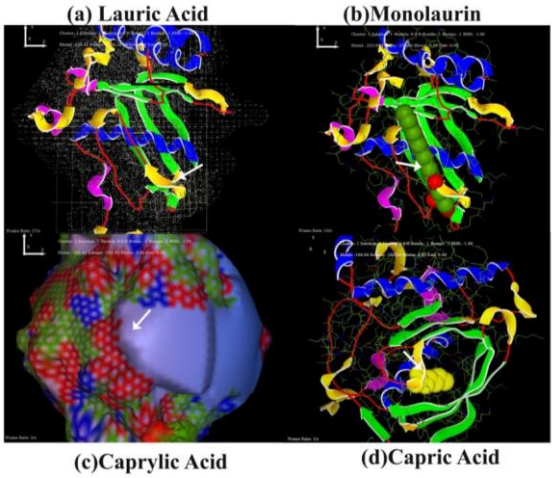

Fig.5. A screenshot from docking of *C.difficile* PDF protein with medium chain fatty acids and monolaurin. Different binding modes of are shown. Ligand binding site was found using ligand receptor interaction tool of Discovery Studio tool.

V. CONCLUSION

Antibiotic resistant pathogenic bacterial strains, which evolve from time to time, have posed a great threat to the human community. Therefore, in order to find out solutions to combat these pathogens, discovery of more potential drug targets is essential. In this study, peptide deformylase, because of its vital role most pathogenic bacteria, was chosen as a target and *in silico* analysis of fatty acids, derived from coconut oil and monolaurin, was carried out against peptide deformylase. The results reveal that these ligands have an efficient binding affinity and certainly could be used for future drug designing.

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