ECA 2021

sciforum

Conference Proceedings Paper

Docking and Molecular Dynamics Simulation Study of Plant Origin Antifungal Peptides with Fungal Protein of Plant Pathogen *Fusarium oxysporum* †

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† Presented at the 4th International Conference nanoFIS 2020—Functional Integrated nano Systems, Graz, Austria, 2–4 November 2020.

Received: date; Accepted: date; Published: date

Abstract: Emerging infectious diseases (EID) are serious problems caused by fungi in humans and plants and are examine as a warning to world food security. Plant origin antifungal peptides are biologically active peptides. They play a vital role in the primary host defense against microbial invasion. The *Fusarium oxysporum*species complex includes phytopathogenic & non-pathogenic strains and is usually found in the soil. *F. oxysporum* has gained significant attention from plant pathologists for more than a century owing to economic losses. Protein-peptide interactions (PPI) play an essential role in living beings and their structural characterization. The current study discusses the molecular interaction study of plant antifungal peptides with the target fungal protein of *F. oxysporum.* We have already reported 510 plant origin antifungal peptides in the PlantAFP database. Under the current investigation, we have selected 55 amino acids under the amino acid length size criteria of 4-30 that are strictly natural amino acids. The protein-peptide interaction study yielded peptide models. The top 5 models, namely **PHYTO5, PHYTO13, PHYTO28, PHYTO1,** and **PHYTO52**, achieved high cluster density with low RMSD values. The peptide docking study was further validated with simulation studies for thermodynamic properties. **PHYTO5** provided important H-bond involvement with **SER57, GLU59, ARG65, HIS68, GLU74,** and **GLU 87.** This can be used as probable anti-fungal peptide inhibitor against fungi based infectious diseases.

Keywords: Plant host defense peptides; Plant defensins; Innate immunity; Antimicrobial peptides; HDPs; AMP; PhytoAFP; Docking; Molecular dynamics

1. Introduction

Plant disease pandemic originates from a fungus; fungus-like oomycete has changed human history. Fungi constitute many plant pathogens and are the main cause of fatal plant diseases. Food security to maintain population growth is a global concern[1]. Most of the *Fusarium oxysporum* found in soil include both pathogenic and non-pathogenic strains[2]. A significant factor affecting food protection is yield loss due to plant diseases and spoilage during post-harvest storage[3]. Entering the 21st century for nearly two decades, opportunistic fungal infections have become a significant cause of human diseases and economically essential crop losses every year[4]. People are paying more and more attention to the drug resistance of microorganisms to many antibiotics, and the demand for alternative drugs has led people to search for new molecules and drugs constantly. They are seeking to develop new biologically active peptides[5]. Plant-derived antifungal peptides are different biologically active peptides, and they play a vital role in the pri-

mary host defense against microbial invasion. In some cases, plant-derived antifungal peptides (PhytoAFP) may cause morphological, physiological, and molecular damage and kill fungal cells[6].

2. Materials and Methods

The purpose of this study is to evaluate the protein-peptide interaction between plant-derived antifungal peptides and the plant pathogen *Fusarium oxysporum* fungal protein. Protein-peptide interactions (PPI) play robust and functional parts in a living being and its structural characterization[8]. Nowadays, PPI is a hot concern of theoretical and experimental research. Protein-peptide molecular docking is a challenging job for modeling problems[9]. This study discussed the molecular interaction study of plant antifungal peptides with the target fungal protein of *F. oxysporum* using the CABS-dock server module for flexible protein-peptide docking. CABS-dock plays a vital role in peptide folding and binding, and the process is straightforward. In the CABS-dock mechanism, no information about the peptide binding site is needed, but its structure can be used in the simulation process. All the result data provided by the CABS-dock server interface is composed of the 10 highest ranked models. VMD software is used to analyze the final structure of the standard[10]. In this study, the RMSD calculation value described using VMD is described in detail[11]. We analyzed the top ten models and the second 10,000 models obtained in the CABS terminal simulation. For the molecular research, we have extracted 510 plant origin antifungal peptides from the PlantAFP database. In this study, we discussed using the CABS-dock server module to study the molecular interaction between the flexible protein-peptide docking plant-derived antifungal peptide and the fungal target fungal protein *F. oxysporum*. The peptide sequence and PDB id of the target protein of the CABS-dock server are used as input. The sequence needs 4-30 amino acids and consists only of natural amino acids. Our research shows that among the 55 project dataset models, the top 5 project dataset models (such as PHYTO5, PHYTO13, PHYTO28, PHYTO1, and PHYTO52) can be used as antifungal inhibitors

3. Results and Discussion

This study discussed the molecular interaction study of plant antifungal peptides with the target fungal protein of *F. oxysporum* using the CABS-dock server module for flexible protein-peptide docking. Our data set created 55 peptides as ligands and 5OD4 PDB as receptor protein (36-137 AA). According to the CABS-dock server, we use the best-case point of view, and the model with the lowest RMSD ranks first[7]. Therefore, we analyzed our data set in the CABS-doc server and created the top 55 models based on high cluster density and low RMSD. However, listing the models is a very complex and yet unsolved problem. We also selected the top 5 models from these 55 models based on the RMSD value (Table 1). Our research shows that in 5 data set models out of 55 models, may be used as antifungal inhibitors. Our top 5 models **PHYTO5, PHYTO13, PHYTO28, PHYTO1,** and **PHYTO52**, the CABS-dock evaluation study achieved high cluster density with low RMSD.

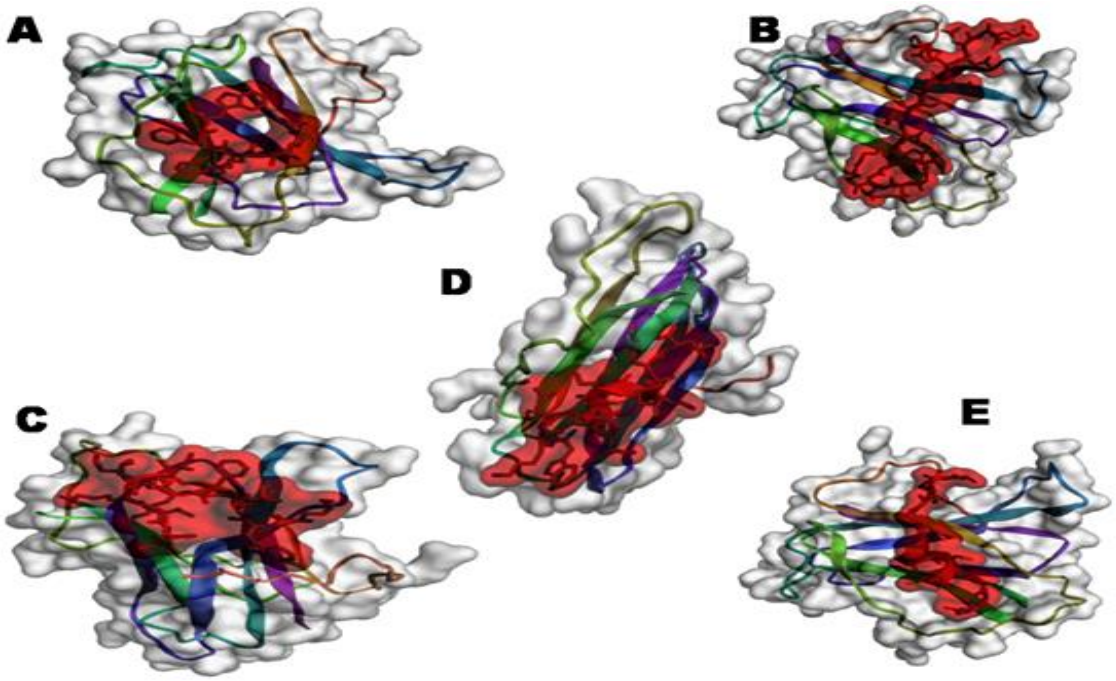

Figure 1. Top-5 models have described in A (PHYTO5), C (PHYTO28) B (PHYTO13), D (PHYTO1), and E (PHYTO52).

Table 1. Top five best models based on high cluster density and low RMSD.

Project Name	Cluster density	Average RMSD	Max RMSD	Number of elements
PHYTO5	**93.2059**	**1.20164**	**19.7809**	**112**
PHYTO13	**57.8178**	**1.43554**	**8.88006**	**83**
PHYTO28	**30.3344**	**1.4505**	**11.5505**	**44**
PHYTO1	**43.0347**	**2.5096**	**18.6126**	**108**
PHYTO52	**38.6859**	**2.68832**	**21.2965**	**104**

The contact map makes it easier to analyze the results and directly understand the molecular interactions of the modeled proteins and it provides an overview in detail for the peptide and protein's residue-residue interaction patterns. Contact map studies indicated the interaction between the receptor and peptide. In the receptor **GLY, PHE TYR, GLU, THR**, and **ALA** interacting residues have been used, while the peptide chain has **ALA, CYS, ARG, LYS,** and **TRP** interacting residue. Our best model project PHYTO5 has an RMSD value of **1.20164**, a good starting point for more precise modeling. We also conducted molecular dynamics studies to understand these plant antifungal peptides' binding mechanism and target protein of *F. oxysporum*. Besides, operations such as RMSD, RMSF, hydrogen bonding, and rotation have been performed. Detailed analysis is performed to understand the stability and changes of protein binding sites during the simulation run. Molecular dynamics has become an important research method, covering millions of atomic-level systems. It's most important to consider hydrogen bonds' properties in drug design because they are essential for drug specificity, metabolism, and adsorption. Molecular dynamics further verify the results produced by the Cabs-dock web server to study thermodynamic properties. We have performed molecular dynamics studies on our top five models **PHYTO5, PHYTO13, PHYTO28, PHYTO1**, and **PHYTO52**, but we describe the analysis of the results of the top model PHYTO5.

3.1. Protein RMSD Findings

The graph shows the evolution of the RMSD of the protein (left Y-axis). First, all protein frames are aligned on the reference frame's backbone, and then the RMSD is calculated based on the atom selection. RMSD analysis can indicate whether the simulation has reached equilibrium, and its fluctuation at the end of the simulation is about some thermal average structure. Our simulation shows that the RMSD value is large, but because the protein is large, it is acceptable, and the

system finally begins to reach equilibrium. Most importantly, during the entire operation, the amino acid at the protein binding site has overlapped with the peptide to form a bond (Figure A).

3.1.1. Ligand RMSD Findings:

Ligand RMSD (right Y-axis) indicates how stable the ligand concerns the protein and its binding pocket. The observed ligand value is significantly lower than the RMSD of the protein. Likely, the ligand has not diffused from its initial binding site (Figure A).

3.2. Protein RMSF:

Root Mean Square Fluctuation (RMSF) can be used to characterize local changes in protein chains. In this figure 3, the peaks represent the protein regions that fluctuate the most during the simulation. Secondary structure components such as alpha helices and beta strands are usually more rigid than the unstructured part of the protein, thus fluctuating less than the loop regions (Figure B).

3.3. Protein Interaction:

The interaction between protein and ligand can be monitored throughout the simulation process. Protein-ligand interactions (or 'contacts') have been classified into four types: Hydrogen Bonds, Hydrophobic, Ionic, and Water Bridges. The stacked bar charts have normalized over the course of the trajectory: for example, a value of 0.7 suggests that 70% of the simulation time, the specific interaction is maintained. In our case, GLU59 shows multiple interactions exceeding 100% (Figure C).

3.4. Ligand RMSF:

The Ligand Root Mean Square Fluctuation (L-RMSF) can be used to characterize the change in the position of the ligand atom. The ligand RMSF may present insights into how ligand fragments interact with the protein and their entropic role in the binding event (Figure D).

3.5. Ligand Protein Contacts:

Detailed schematic diagram of the interaction between ligand atoms and protein residues. This shows that the interaction time that occurred in the selected trajectory (0.00 to 100.00 ns) exceeds 30.0% of the simulation time. In our current findings, **SER57, GLU59, PRO60, ARG65,** and **ILE85** of the receptor fungal protein are responsible in forming multiple interactions with the peptide ligand exceeding 30% contacts (Figure E).

4. Conclusions:

Our study with contact maps supported by simulations for the top model PHYTO5 suggests that SER57, GLU59, ARG65, HIS68, GLU74 and GLU 87 are involve in major hydrogen bond with the peptides. Consideration of hydrogen-bonding properties in drug design is most important because they will strongly influence drug specificity, metabolism, and adsorption[12]. To the best of our knowledge, there is no investigation of molecular interaction study of plant-derived antifungal peptides with target fungal protein of plant pathogen *Fusarium oxysporum*. Our study with contact maps supported by simulations for the top model PHYTO5 suggests that SER57, GLU59, ARG65, HIS68, GLU74, and GLU 87 are significant hydrogen bonds of the peptides.

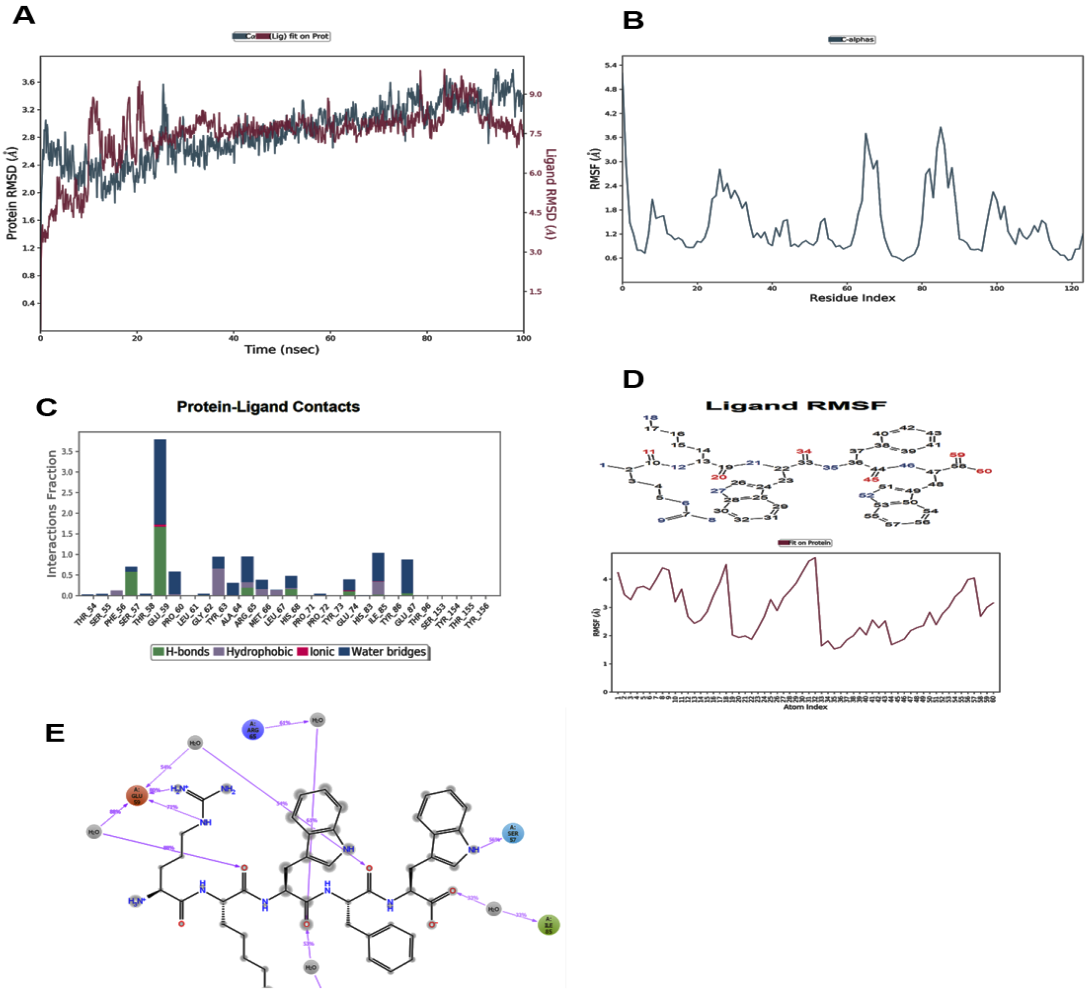

Figure 2. The top-5 models have described in A (Protein-Ligand RMSD), B (Protein RMSF), C (Ligand Protein Contacts), D (Ligand-RMSF), and E (Protein-Ligand contacts).

Author Contributions: A.T. and S.R. collected the data and created the datasets. A.T., S.R., A.T., S.R., and I.P designed and performed the experimental studies.I.P. A.K.S and A.S. The all authors read and approved the final manuscript.

Funding: The author would like to thank the Brno University of Technology in Brno, Czech Republic, CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP) Lucknow, India, and Jawaharlal Nehru University (JNU) for their support. Author (Atul Tyagi) is thankful to the International Mobility of Researchers of Brno University of Technology (Project registration number CZ.02.2.69/0.0/0.0/16_027/0008371). The Brno University of Technology in Brno, Czech Republic provided financial support.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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