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In silico analysis of 1H-1,2,4-Triazole, 1-octadecanoyl Revealed anti-oomycete Nature against *Phytophthora infestans* causing Late Blight of Potato

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ABSTRACT: Late blight of potato incited by *Phytophthora infestans* is one of the main obstacles for potato and tomato production in India. Co-evolution of pathogen with fungicides made the management of late blight a challenging task throughout the world. The main aim of this study was to elucidate the role of 1H-1,2,4-Triazole, 1-octadecanoyl, identified from the zone of inhibition of di-trophic interaction of *Bacillus subtilis* NM261 and *Phytophthora infestans,* against *Phytophthora infestans* target proteins. For molecular docking, the target proteins such as RxLR effector protein Avr1, Elicitin and Glucanase inhibitor protein 3 were selected based on their role in pathogenesis of *P. infestans*. The three-dimensional structures of target proteins were developed using software; SWISS-MODEL, Phyre2 and ROBETTA. The hypothetical crystal structures were validated using Ramachandran plot before being docked. The fungicide molecule, metalaxyl was used as a positive control. The results revealed that the biomolecule 1H-1,2,4-Triazole, 1-octadecanoyl could bound with all the target proteins and had higher binding energy values in comparison with fungicide molecule, metalaxyl. Thus, these findings confirmed the anti-oomycete nature of 1H-1,2,4-Triazole, 1-octadecanoyl against *P. infestans* through molecular docking.

Keywords: *Phytophthora infestans*, 1H-1,2,4-Triazole, 1-octadecanoyl, molecular docking, *Bacillus subtilis*, endophyte, GC-MS.

INTRODUCTION

Potato is the world largest vegetable crop with an annual production of 300 million metric tonnes (International Potato Center, 2021). However, the productivity of potato is hampered due to nutrients imbalance, diseases, pest and nematodes. Among the major diseases in potato, late blight caused by an oomycete, *P. infestans* is recognized as one of the most widespread and destructive disease, and a major production constraint to potato worldwide. In this situation, several approaches have been made to control the late blight of potato. So far there was no feasible strategies currently available to completely protect these plants against this deadly menace. Chemical fungicide-based disease control leads to higher amount of fungicide intake which results in resistant to microbial degradation, eventually the development of resistance in the pathogen (Fernández-Ortuño *et al.*, 2006).

Azoxystrobin and biomolecule, chaetoglobosin applied potato plants showed the increased resistance towards late blight of potato pathogen, *P. infestans* by increasing the levels of peroxidase, poly phenol oxidase and superoxide dismutase (Srinivasan *et al*., 2016). In this juncture, microbial biocontrol agents can pave the way for the biological control of plant diseases through their bioactive secondary metabolites. Recent advances in molecular modelling and docking would allow to find out the potential anti-oomycete biomolecules of microbial origin and it will help toannotate potential protein target sequences of the *P. infestans* that play a key role in the pathogenicity resulting in the suppression of late blight pathogen (Isyaku *et al.*, 2020). Considering the significance, *in silico* analysis was carried out to dock the biomolecule, 1H-1,2,4-Triazole, 1-octadecanoyl against three target proteins of *P. infestans* to discover the anti-oomycete action.

MATERIALS AND METHODS

Identification of protein targets of *P. infestans* and molecular modelling: The three target proteins of *P. infestans viz*, RxLR effector protein Avr1, Elicitin and Glucanase inhibitor protein 3 were selected based on their role in pathogenesis. The amino acid sequences

were retrieved from the online database UniProt. As there is no crystal structure of the proteins was available in Protein Data Bank Database, we went for the modelling of 3D structure of the target proteins using SWISS-MODEL (Method: Rigid-body assembly), Phyre2 (Method: Profile based alignment), and ROBETTA (Meta server, https://robetta.bakerlab.org/) based on query coverage performance and percentage identity of the proteins, which was obtained from BLASTp search.

Structure Validation: The quality of the modelled protein structure was assessed through Ramachandran plot of the PROCHECK tool from the Structural Analysis and Verification Server (SAVES, Meta server, https://saves.mbi.ucla.edu/) to understand the phi and psi scatter of amino acid residues. The energy minimization for proteins and loop building for residues in disallowed regions were performed using Swiss PDB Viewer.

Ligand preparation and analysis: Two ligand molecules *viz*, 1H-1,2,4-Triazole, 1-octadecanoyl (biomolecule identified from zone of inhibition during interaction of *Bacillus subtilis* NM261 and *P. infestans*) and metalaxyl (reference ligand molecule)were retrieved from the Pub Chem database in Structure Data File (SDF) format. OpenBabel software (O'Boyle *et al*., 2011) was used to convert compounds from SDF to Protein Data Bank (PDB) file format.

Virtual screening and molecular docking: For performing molecular screening, the Auto Dock Vina module in PyRx 0.8 was used (Dallakyan and Olson, 2015). PyRx was used to identify the potential compound inhibitors against the target proteins of *P. infestans*. The energy minimization process was achieved via the field of universal force of optimization process with 200 steps. The Computed Atlas Topography of Proteins CASTp 3.0 server was used to find binding site pockets for the targets (Tian *et al.*, 2018). Docked conformations of protein-ligand complexes were imported into BIOVIA Discovery studio client 2021. Interactions that are observed are logged and exported as photographs. The H-bond surface receptor was used to highlight the ligand-binding site. Different colours were assigned to the receptor, ligand, and interacting atoms to distinguish them.

RESULTS AND DISCUSSION

Crystal structures of *P. infestans* protein targets: The crystal structures of RxLR effector protein Avr1, Elicitin and Glucanase inhibitor protein 3 was modelled using SWISS, Phyre2 and ROBETTA software, respectively (Fig. 1).

Molecular docking: Molecular docking results revealed that the biomolecule 1H-1,2,4-Triazole, 1-octadecanoyl was effectively bound with all the target sites of *P. infestans* used in this study (Fig. 2). The binding energy values, amino acid interactions and type of bonding were represented in Table 1. Plant pathogens frequently use a variety of effectors to aid infection. Elicitins are found in oomycetes, most notably in the species of *Phytophthora* and *Pythium*. They serve an important biological process in oomycetes as sterol transporters. The highest binding affinity (-4.6 kcal/mol) of 1H-1,2,4-Triazole, 1-octadecanoyl with elicitin was obtained. By binding the biomolecule 1H-1,2,4-Triazole, 1-octadecanoyl with elicitin could inhibit the sterol transport from plants which affect the life cycle of *P. infestans* (Ponchet *et al*., 1999). Numerous RXLR effectors are secreted by *P. infestans*, which alter host defence and therefore pave the way for effective invasion. Colonization and callous deposition in host plants are inhibited by RXLR effector AVR1, a virulence factor. The binding energy of 1H-1,2,4-Triazole, 1-octadecanoyl with RXLR effector protein Avr1 was-4.2 kcal/mol, could block the functions of RXLR in host tissues like colonization of *P. infestans* (Du *et al*., 2015). Glucanase inhibitor proteins (GIP) which are secreted by *P. infestans*, bind and block the action of plant extracellular endo-1,3-glucanases during the invasion of host plants. In the present study, the biomolecule 1H-1,2,4-Triazole, 1-octadecanoyl was able to bound with the glucanase inhibitor protein 3 with binding energy of -3.7 kcal/mol, which could affect the function of GIP and enables the action of plant glucanase enzymes on *P. infestans* cell wall (Damasceno *et al.*, 2008). Interestingly, the biomolecule 1H-1,2,4-Triazole, 1-octadecanoyl had comparatively higher binding affinity to elicitin, RXLR effector AVR1 and GIP3 than the positive control, metalaxyl, an oomycete fungicide molecule (Fig. 3). Thus, these findings provided a clearer understanding of the role of 1H-1,2,4-Triazole, 1-octadecanoyl in the control of *P. infestans*.

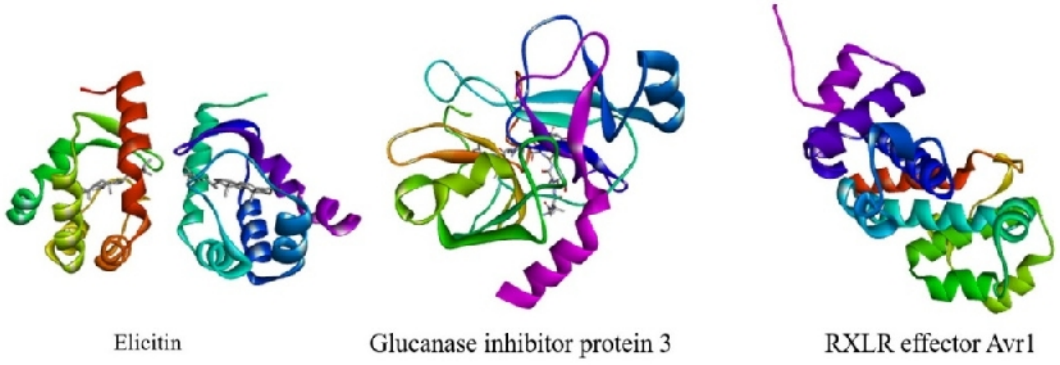

Fig. 1. Three-dimensional structures of *P. infestans* target proteins.

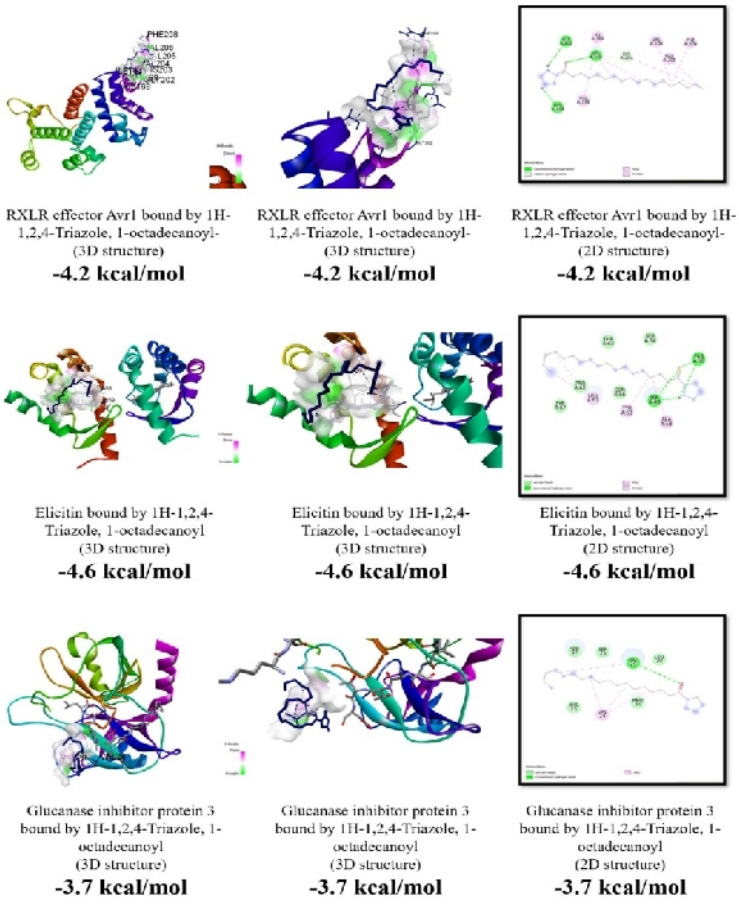

Fig. 2. Images showing 1H-1,2,4-Triazole, 1-octadecanoyl binding with *P. infestans* target proteins; RXLR effector AVR1, Elicitin and Glucanase inhibitor protein 3.

Table 1: Results of molecular docking; 1H-1,2,4-Triazole, 1-octadecanoyl and metalaxyl interaction with active binding sites of *P. infestans* protein targets.

Compounds	Protein targets of *P. infestans* with Uni Prot ID	Binding affinity (Kcal/mol)	Interacting amino acids	Type of bonding
1H-1,2,4-Triazole, 1-octadecanoyl	Elicitin Q01905 (Q01905_PHYIN)	-4.6	TYR53 SER54 ALA58 THR59 SER60 LEU61 PRO62 THR63 GLN66 TYR67	Pi-Alkyl van der Waals Pi-Alkyl Conventional Hydrogen Bond Conventional Hydrogen Bond Alkyl van der Waals van der Waals van der Waals van der Waals
	RXLR effector protein Avr1 D0NVB5 (AVR1_PHYIT)	-4.2	ILE164 HIS168 VAL198 GLY202 HIS203 VAL204 VAL205 VAL206 PHE208	Pi-Alkyl Conventional Hydrogen Bond Alkyl Conventional Hydrogen Bond Carbon Hydrogen Bond Conventional Hydrogen Bond Pi-Alkyl Alkyl Pi-Alkyl
	Glucanase inhibitor protein 3 B1AC88 (GIP3_PHYIN)	-3.7	HIS23 VAL24 SER25 VAL29 LEU30 PRO36 SER37	van der Waals Alkyl van der Waals Conventional Hydrogen Bond van der Waals van der Waals van der Waals
	Elicitin Q01905 (Q01905_PHYIN)	-4.4	GLY52 TYR53 SER54	Pi-Donor Hydrogen Bond van der Waals van der Waals

Metalaxyl			THR57 ALA58 THR59 SER60 THR63 GLN66	van der Waals van der Waals Conventional Hydrogen Bond Conventional Hydrogen Bond van der Waals Conventional Hydrogen Bond
	RXLR effector protein Avr1 D0NVB5 (AVR1_PHYIT)	-4.0	ARG197 LEU200 GLY202 HIS203	Pi-Alkyl Carbon Hydrogen Bond van der Waals Conventional Hydrogen Bond
	Glucanase inhibitor protein 3 B1AC88 (GIP3_PHYIN)	-3.7	THR21 HIS23 VAL24 SER25 LEU28 VAL29 PRO36 SER37	van der Waals Carbon Hydrogen Bond van der Waals van der Waals van der Waals Pi-Cation van der Waals Conventional Hydrogen Bond

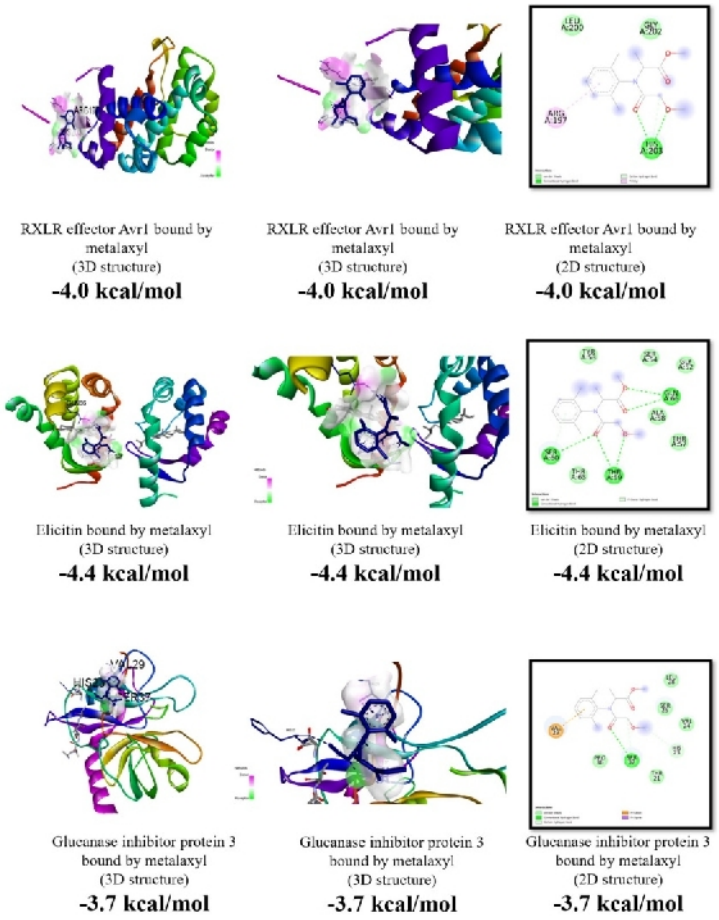

Fig. 3. Images showing metalaxyl binding with *P. infestans* target proteins; RXLR effector AVR1, Elicitin and Glucanase inhibitor protein 3.

CONCLUSION

In silico analysis of biomolecule 1H-1,2,4-Triazole, 1-octadecanoyl with *P. infestans* target proteins, elicitin, RXLR effect or AVR1 and GIP3 revealed that the biomolecule could bound the target molecules with binding energy of -4.6 kcal/mol, -4.2 kcal/mol and -3.7 kcal/mol respectively. Also, the biomolecule had comparatively higher binding affinity than metalaxyl, which confirmed the anti-oomycete nature of 1H-1, 2, 4-Triazole, 1-octadecanoyl against *P. infestans*. Further, the validation of 1H-1, 2, 4-Triazole, 1-octadecanoyl in wet lab experiments and in field conditions are required for its effective deployment against late blight of potato.

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Conflict of Interest. None.

REFERENCES

Dallakyan, S. and Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. In Chemical biology. Humana Press, New York, NY, p. 243-250.

Damasceno, C. M., Bishop, J. G., Ripoll, D. R., Win, J., Kamoun, S. and Rose, J. K. (2008). Structure of the glucanase inhibitor protein (GIP) family from *Phytophthora* species suggests coevolution with plant endo-β-1, 3-glucanases. *Molecular Plant-Microbe Interactions, 21* (6): 820-830.

Du, Y., Mpina, M. H., Birch, P. R., Bouwmeester, K. and Govers, F. (2015). *Phytophthora infestans* RXLR effector AVR1 interacts with exocyst component Sec5 to manipulate plant immunity. *Plant Physiology, 169* (3): 1975-1990.

Fernández-Ortuño, D., Pérez-García, A., López-Ruiz, F., Romero, D., De Vicente, A. and Torés, J. A. (2006). Occurrence and distribution of resistance to QoI fungicides in populations of *Podosphaera fusca* in south central Spain. *European Journal of Plant Pathology, 115*(2): 215-222.

International Potato Center (2021). CIP Annual Report 2020. Build, Innovate, Transform: Collaborative solutions for global challenges. Lima, Peru. International Potato Center. 40.

Isyaku, Y., Uzairu, A. and Uba, S. (2020). QSAR and molecular docking studies of novel 2, 5-distributed-1, 3, 4-thiadiazole derivatives containing 5-phenyl-2-furan as fungicides against *Phythophthora infestans*. *Beni-Suef University Journal of Basic and Applied Sciences, 9*(1): 1-12.

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T. and Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics, 3*(1): 1-14.

Ponchet, M., Panabières, F., Milat, M. L., Mikes, V., Montillet, J. L., Suty, L., Triantaphylides, C., Tirilly, Y. and Blein, J. P. (1999). Are elicitins cryptograms in plant-Oomycete communications?. *Cellular and Molecular Life Sciences, 56*(11): 1020-1047.

Srinivasan, V. M., Jebaraj, M. D. and Krishnamoorthy, A. S. (2016). Induction of resistance against late blight disease on potato by azoxystrobin and chaetoglobosin biomolecules. *Journal of Pure and Applied Microbiology, 10*(4): 3023-3028.

Tian, W., Chen, C., Lei, X., Zhao, J. and Liang, J. (2018). CASTp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Research, 46*(W1): W363-W367.

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