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Molecular Docking Reveals 2,4-Di-tert-butylphenol as a Novel Biomolecule of *Bacillus atrophaeus* Origin for the Management of *Phytophthora infestans*

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ABSTRACT: The broad spectrum anti-oomycetes activity of biomolecules produced by bacterial endophyte *Bacillus atrophaeus* NMB01 were explored in order to combat *Phytophthora infestans*, the incitant of late blight of potato. Molecular modelling and docking were performed to explore the interaction of 2,4-Di-tert-butylphenol, N-Nitrosopyrrolidine and tetradecanoic acid produced by *B. atrophaeus* NMB01 against *P. infestans* with its protein targets autophagy-related protein 8, cytochromec oxidase subunit 1, calmodulin and b ZIP transcription factor 1. The commercially available fungicide mandipropamid was used as a positive control. *In silico* analysis revealed that 2,4-Di-tert-butylphenol had the highest binding affinity for the target calmodulin (-6.5 kcal/mol) in comparison with the fungicide mandipropamid. We conclude from the present study that biomolecule 2,4-Di-tert-butylphenol can be explored for the anti-oomycete activity.

Keywords: *Phytophthora infestans*, *Bacillus atrophaeus* NMB01, molecular docking, 2,4-Di-tert-butylphenol, anti-oomycete activity.

INTRODUCTION

Potato is an important vegetable and staple food crop around the world because of its high yield per unit area and itsability to act as a nutrient and mineral reservoir. Unfortunately, its productivity was declined due to its susceptibility towards pest and diseases. The single oomycete *P. infestans* causing devastating yield losses of up to 100% (Nowicki *et al*., 2012). In this juncture, several strategies like cultural practices, use of chemical fungicides and biocontrol agents have been deployed to manage late blight disease. Of which, the effectiveness of chemical fungicides was lowered due to its negative impact on the environment and the emergence of resistance in the pathogen (Oyesola *et al*., 2021). Therefore eco-friendly employment of microorganisms in the biological control of pathogens replaces chemical fungicides and serves as a separate line of defense (Shailbala and Kumar 2017). Bacterial endophytes of the genus *Bacillus* are known to produce antimicrobial biomolecules that are the potent inhibitors of phytopathogens. The bacterial antagonist *B. atrophaeus* had inhibitory activity against various phytopathogens through their secondary metabolites (Huang *et al*., 2015; Mu *et al*., 2020). The in vitro and in vivo screening of putative bioactive chemicals is extremely challenging and time-consuming. Consequently, molecular modelling and docking might simplify the identification of potent anti-oomycete biomolecules and it will aid in characterization of protein target sequencesof *P. infestans* involved in pathogenicity. In this regard molecular docking of biomolecules 2,4-Di-tert-butylphenol,N-Nitrosopyrrolidine and tetradecanoic acid produced by *B. atrophaeus*NMB01 against four protein targets of *P. infestans* were done to discover the biomolecule with anti-oomycete action.

MATERIALS AND METHODS

Molecular modelling and structure validation of target proteins of *P. infestans.* The protein targets which are highly essential for the growth, development, survival and pathogenesis of *P. infestans* were choosen based on literature search. The protein sequences of target proteins were acquired from the Uniprot database. FASTA sequence of the target proteins were subjected to BLAST to compare the protein query percentage in the protein database. Molecular modelling was performed using SWISS-MODEL (Method: Rigid-body assembly) and ROBETTA (Meta server, https://robetta.bakerlab.org/) in accordance with

query coverage performance and percentage identity obtained from Blastp.

In order to assure that the modeled protein targets have a high level of quality, the Ramachandran plot of the PROCHECK tool on the Structural Analysis and Verification Server (SAVES, Meta server) (https://saves.mbi.ucla.edu/) was used to validate the models of the protein targets. This plot displays which residues are located in regions that are preferred or allowed. The Swiss PDB Viewer (http://www.expasy.org/spdbv/) was utilized for the purpose of energy minimization in modeled proteins as well as loop creation for residues lying in disallowed regions.

Ligand preparation. The two dimensional structure of three ligands 2,4-Di-tert-butylphenol, N-Nitrosopyrrolidine and tetradecanoic acid produced by *B. atrophaeus* NMB01 and the reference ligand molecule mandipropamid as a positive control were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) as SDF format. Through Open Babel software, 2D structure was converted to 3D structure in PDB format.

Molecular docking. Molecular docking was carried out using the Auto Dock Vina module in PyRx 0.8 (Dallakyan and Olson 2015). Targets were turned into protein macromolecules using PyRx. A 200-step first-order derivative optimization approach using commercial molecular mechanics parametersthe Unified Force Field were used to minimize all ligand structures in order to free up troublesome angles (UFF). The targets binding site pockets were located using the CASTp 3.0 server from the Computed Atlas Topography of Proteins (Tian *et al*., 2018). When a rigid receptor was used, ligands could yield flexible conformations and orientations with an exhaustiveness value of 8.

Docked complex visualization. The BIOVIA Discovery studio client 2021 was updated with the docked conformations of protein-ligand interactions. Observed interactions are registered and then exported as pictures for further analysis. The H-bond surface receptor was utilized so that the ligand binding site could be highlighted more clearly. In order to differentiate between the receptor, the ligand, and the interacting atoms, each one was given a distinct colour.

RESULTS AND DISCUSSION

Homology and Ab initio modelling. The 3D structure of the target proteins autophagy-related protein 8 and cytochrome c oxidase subunit 1 were modelled using SWISS model server, which had 77.39% and 65.97% sequence identity between template and modelled structure, respectively (Fig. 1). Similarly, for calmodulin and bZIP transcription factor 1, crystal structure was modelled with ROBETTA programme with 65% and 50% confidence score, respectively **(**Fig. 1).

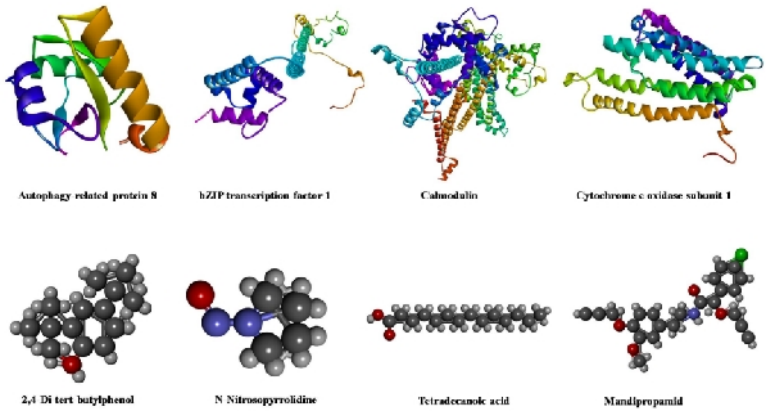

Fig. 1. The 3D structure of the target proteins and ligand molecules.

Structure validation. Structural validation of modelled 3D structure of the targetsautophagy-related protein 8, cytochrome c oxidase subunit 1, calmodulin and bZIP transcription factor 1 through Ramachandran plot had 96%, 93.3%, 91% and 84% of amino acid residues in the most favoured region, respectively.

Virtual screening and molecular docking. *Insilico* docking studies between four target proteins of *P. infestans* (autophagy-related protein 8, cytochrome c oxidase subunit 1, calmodulin and bZIP transcription factor 1) and three ligand molecules (2,4-Di-tert-butylphenol, N-Nitrosopyrrolidine and tetradecanoic acid) revealed that 2,4-Di-tert-butylphenol had the highest docking score of -6.5 kcal/mol which was shown in the heat map (Fig. 2). The binding of 2,4-Di-tert-butylphenol with four target proteins were compared with reference ligand molecule mandipropamid (Fig. 3). The maximum binding energy of 2,4-Di-tert-butylphenol (-6.5 kcal/mol) with the target protein calmodulin could inhibit the communication between plant-microbe interaction and reduce the mRNA levels in the pathogen (Pieterse *et al*., 1993). Similarly, binding of 2,4-Di-tert-butylphenol with cytochrome c oxidase subunit 1(-6.2 kcal/mol) could impede the supply of ATP essential for pathogenicity by disrupting the electron transport process (Pan *et al*., 2018). Binding of2,4-Di-tert-butylphenol with bZIP transcription factor 1 and autophagy-related protein 8 could block the appressoria formation by inhibiting the zoospore motility (Blanco *et al*., 2005) and virulence of *P. infestans* (Chen *et al*., 2017). In the previous study, *in silico* analysis was done

to reveal the anti-oomycete nature of 1H-1,2,4-Triazole, 1-octadecanoyl produced during ditrophic interaction of *B. subtilis* NM261 and *P. infestans*. Interacting amino acids showing H-bonding, hydrophobic interactions, Van der Waals force, Pi-Alkyl and carbon hydrogen bonds between ligands and target proteins were depicted in Table 1. As the ligand binds to four separate target proteins, resistance in the pathogen is unlikely to emerge because of the various modes of action. Thus, docking results confirmed that 2,4-Di-tert-butylphenolcan be used to manage *P. infestans*.

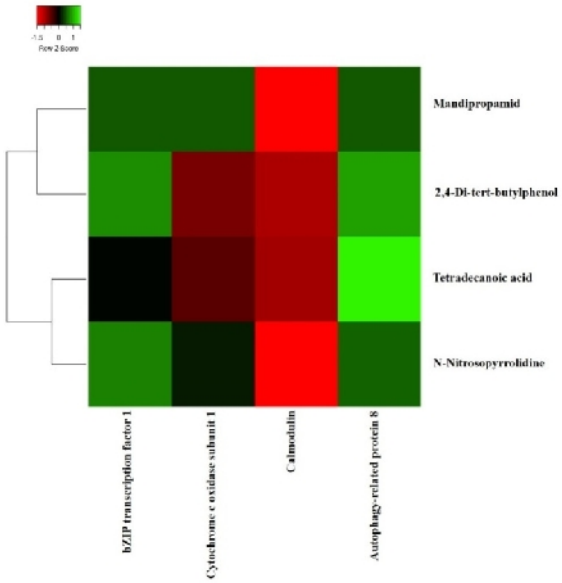

Fig. 2. Heatmap based on binding energy among four target proteins of *P. infestans* and three ligand molecules.

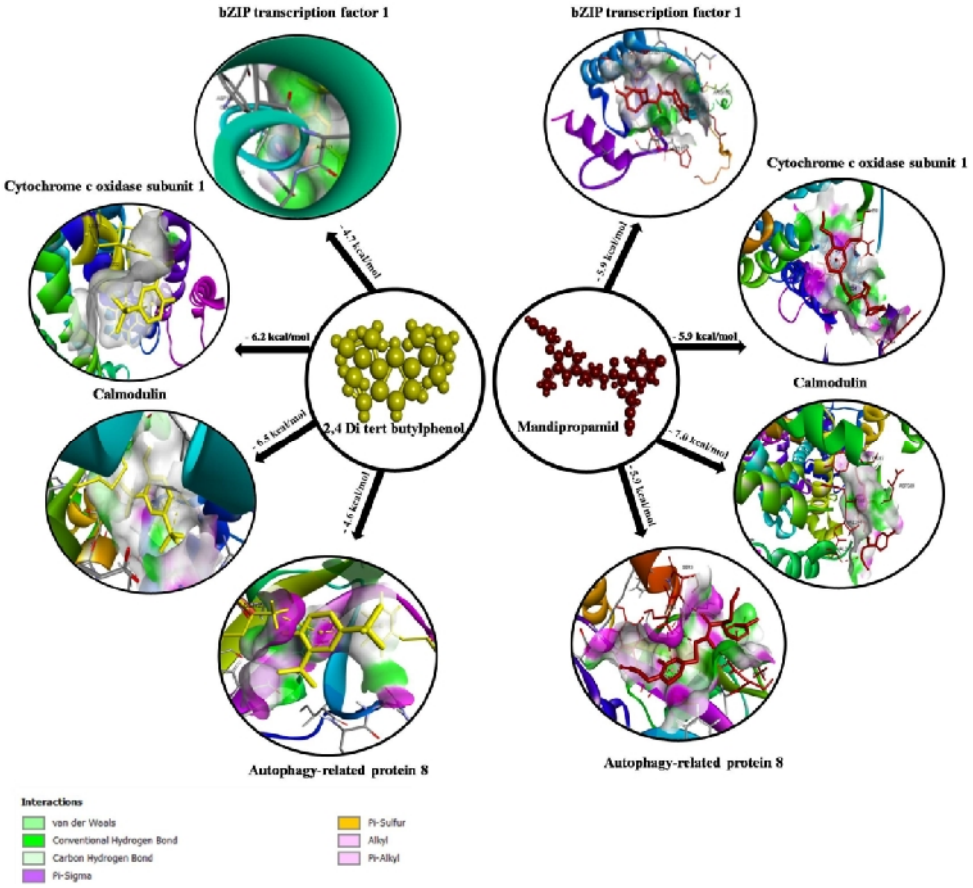

Fig. 3. Illustrative diagram depicts the protein-ligand interaction of the biomolecule 2,4 Di tert butylphenol and the reference ligand molecule mandipropamid.

Table 1:Interacting amino acids between ligands and target proteins

Biomolecules	Receptors	Etotal kcal/mol	Interacting amino acids	
			H-bonding	Other interactions
2,4-Di-tert-butylphenol	Autophagy-related protein 8	-4.6	-	ASN 81, ASP 36, PHE 114, SER 2, LYS 5, GLU 33, THR 107, TYR 105
	bZIP transcription factor 1	-4.7	-	ALA 121, ARG 122, ARG 128, TYR 123, GLN 127, ASP 125, GLN 120
	Calmodulin	-6.5	-	LEU 516, ARG 523, ARG 519, TYR 624, LEU 621, LEU 625, PRO 259, ALA 622, GLU 178, MET 230, PHE 258, ARG 257
	Cytochrome c oxidase subunit 1	-6.2	-	PHE 222, ILE 226, TYR 223
N-Nitrosopyrrolidine	Autophagy-related protein 8	-3.9	-	CYS 32, ILE 31, ILE 106, TYR 105, GLU 33, SER 2, THR 107
	bZIP transcription factor 1	-3.8	TYR 123, ASP 125	ARG 122, ARG 124, ALA 121, GLN 127, ARG 128, LYS 126
	Calmodulin	-4.9	TRP 393, ASP 386	ASP 389, GLY 392, VAL 394, TYR 387, GLY 388, HIS 341, GLU 344, GLN 340, ARG 347
	Cytochrome c oxidase subunit 1	-4.1	GLY 227, TYR 223	LYS 228, TRP 225, ILE 226, PRO 166, PHE 224, TYR 234, PHE 91
Tetradecanoic acid	Autophagy-related protein 8	-3.6	ILE 106, GLU 33, ILE 31	CYS 32, THR 107, PHE 114, TYR 105, SER 2, LYS 5, ASN 80
	bZIP transcription factor 1	-4.6	ARG 219, MET 215	PHE 173, GLY 220, ILE 221, VAL 213, LEU 218, ALA 214, LEU 224, LEU 228, PHE 209, VAL 172, VAL 169
	Calmodulin	-5.3	GLU 691, GLU 690	GLU 714, GLY 694, THR 613, TYR 717, ARG 641, LYS 718, ALA 612, VAL 608, SER 721, ALA 722
	Cytochrome c oxidase subunit 1	-5.0	-	ILE 229, PHE 222, ILE 226, HIS 242, LEU 238, GLY 239, TYR 234, PRO 235, TYR 223
Mandipropamid	Autophagy-related protein 8	-5.9	ASP 36, SER 3	ASP 99, LYS 5, PHE 114, ALA 35, ASP 42, SER 2, LYS 34, LYS 46, GLU 33, ILE 106, THR 107, TYR 105
	bZIP transcription factor 1	-5.9	-	ARG 160, THR 161, ARG 207, ASN 162,GLN 163, SER 208, PRO 165,PHE 209, VAL 169, THR 168,ASP 212,HIS 211, THR 210
	Calmodulin	-7	HIS 341	GLN 340, TYR 387, GLY 388, TRP 393, THR 337, VAL 394, ASP 399, VAL 398, ASN 403, GLY 404, ARG 345, ARG 338, GLU 344, ASP 389, PRO 390, ARG 347
	Cytochrome c oxidase subunit 1	-5.9	PHE 224	SER 90, ALA 89, GLN 86, TRP 225, LYS 228, PRO 166, TYR 223, PHE 91, TYR 234, GLY 227, ARG 233

CONCLUSION

The findings of this study validated the anti-oomycete activity of biomolecules produced by *B. atrophaeus* NMB01 against *P. infestans*. Based on the binding energies of the protein-ligand interactions, it was identified that the ligand 2,4-Di-tert-butylphenol acted as potent inhibitors of the target proteins autophagy-related protein 8, cytochrome c oxidase subunit 1, calmodulin and bZIP transcription factor 1 of *P. infestans*. As a result, 2,4-Di-tert-butylphenol have the potential to be investigated as novel biomolecule for the control of *P. infestans.*

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Conflict of Interest. None.

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