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Biological control and plant growth promotion using co-culture of *Trichoderma* spp. and its molecular docking studies against bacterial leaf blight of rice

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Abstract
Rice disease bacterial leaf blight (BLB) is one of the most destructive diseases throughout the world. The present work is focused on plant growth promoting fungi (PGPF) *Trichoderma harzianum*(PGT4), *Trichoderma reesei*(PGT5), and *Trichoderma reesei*(PGT13), and co-culture of *Trichoderma* spp.(PGTA). Molecular docking studies were carried out to support the antibacterial activity of PGTA against *Xanthomonas oryzae* pv. *oryzae*(Xoo). Docking implies a strong binding affinity of ligand 8 and ligand 10 against both the target proteins suggest that both ligands can block the activity of proteins. Overall, *Trichoderma* spp. can be used as biocontrol and plant growth promotion in field conditions.

Keywords: ligand, *trichoderma* spp., co-culture, rice, bacterial leaf blight, docking

Introduction

Rice is known globally as a major food crop for more than 3.5 billion people as their staple food and calorie source (Chukwu *et al.*, 2019) [1]. The rice disease bacterial leaf blight (BLB) is one of the significant biotic stresses and most destructive throughout the world, causing yield losses up to 50%. It is caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), which is gram-negative bacteria (Singh *et al.*, 2018) [15]. Considering the severity of the disease, different management strategies have to be initiated that are not only environmentally-friendly but also cost-effective. The application of chemical fertilizers has negative impact on the environment and food (Mueen *et al*., 2014) [7].

Biological control refers to the purposeful utilization of introduced or resident living organisms, other than disease-resistant host plants, to suppress the activities and populations of one or more plant pathogens (Ji *et al.*, 2008) [5]. Biological control is an ecological-conscious, cost-effective, and sustainable alternative in BLB management (Palaniyandi *et al*., 2006) [10]. Control of plant disease by the use of antagonistic microorganisms can be an effective means. Interaction between bio control agents and plant pathogens has been studied extensively, and the application of bio control agents to protect some commercially important crops is promising (Shyamala & Sivakumaar 2012) [13].

If a strain has the ability to influence directly or indirectly the growth of a plant it is called a plant growth promote. *Trichoderma* spp. is one among the significant fungi which is filamentous, found in agricultural habitats. It is also found in rhizosphere because of its colonising ability and can also progress in different type of soils from distinct geographic areas (Naznin *et al*., 2014) [8].

Non-pathogenic, filamentous, saprophytic rhizosphere fungi that significantly enhance the growth of plants are known as plant growth-promoting fungi (PGPF). PGPF such as *Fusarium*, *Penicillium*, *Phoma*, and *Trichoderma* have been frequently studied and evaluated for their high suppressive abilities against various plant diseases due to direct antagonism against soil-borne pathogens or by inducing systemic resistance in the plant (Ghazanfar *et al*., 2018) [2]. Currently in drug designing and in macro molecular structure and interaction studies computational docking plays an important role. Computational docking is typically performed by employing a simpler force field and exploring a wider region of conformational space (Huey *et al.*, 2007) [4]. The present Study was focused on the effect of *Trichoderma* spp. on Xoo. The docking studies were carried out in order to know the binding affinity of the ligand molecule of *Trichoderma* spp. against the target protein of Xoo.

Materials and Methods

Ligands and receptor preparation

All ligands listed in Table 1 obtained from the Pubchem database were based on an extensive literature review. These ligands were converted to PBD format using Openpable (O'Boyle *et al.*, 2011) [9]. In our previously published data, we have done the molecular characterization of Trichoderma spp. which are used for field studies. We have also carried out extraction of secondary metabolites produced from co-culture of *Trichoderma* spp. which were identified by GC-MS analysis and subjected for its antibacterial activity against Xoo (Shobha *et al.*, 2020) [12], where the compounds present in the crude extract contributed to the inhibition of Xoo, based on these results, we have carried docking studies.

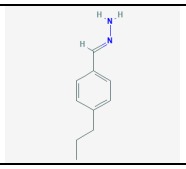
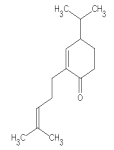
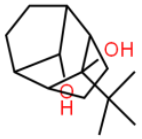
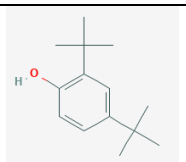
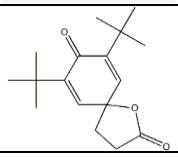
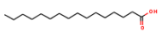
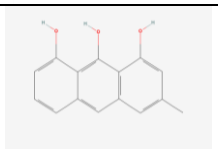
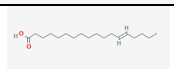
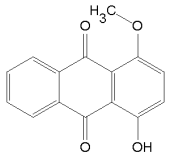
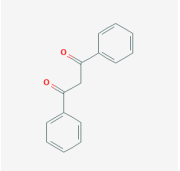
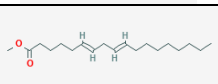
3D Crystal structure of the target antimicrobial protein molecules such as rice cell wall degrading esterase LipA (pdb ID-3H2G) and D-alanine-D-alanine ligase A from Xoo (PDB ID-3R5F) obtained from the RCSB protein data bank. Autodock is an automatic docking software used for docking studies to determine ligands binding affinity against these target receptor molecules. All ligands and receptor proteins were prepared before docking (Harish *et al*, 2013) [3].

Docking of ligands against Antimicrobial proteins

Using MGL tools, Grid maps (Huey *et al.*, 2007) [4]. Spacing adjusted for receptors was to provide enough space for ligands to move free during docking (Seeliger & Groot 2010) [11].

Autodock vina (Trott & Olson 2010) [16] were used to dock and analyze binding free energy between the molecules Ligand-receptor. The docking method followed was carried out with slight modification (Harish *et al*, 2013 & Morris *et al.*, 1998) [3, 6].

Table 1: A list of ligands used in docking analysis against target proteins.

SL. No.	RT (mins)	Name of the compound Sample A (4,5,13)	Molecular formula	Molecular weight	Structures
1	10.71	Benzaldehyde, 4-propyl	$C_{10}H_{12}O$	148	
2	18.11	1-[2-Methyl-2-(-4-methyl-3-pentenyl) cyclopropyl] ethanol	$C_{12}H_{22}O$	182	
3	32.47	9-t-Butyltricyclo [4.2.1.1(2,5)]decane-9,10-diol	$C_{14}H_{24}O_2$	224	
4	7.94	Nonanal	$C_9H_{18}O$	142	
5	14.03	Phenol, 2,4,-di-tert-butyl	$C_{14}H_{22}O$	206	
6	21.55	1-Oxa-spiro[4,5]deca-6,9-diene-2,8-dione, 7,9-di-tert-butyl	$C_{17}H_{24}O_3$	276	
7	22.28	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	
8	23.91	Chrysophanic acid anthranol	$C_{15}H_{12}O_3$	240	
9	25.58	Trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	
10	26.95	1-Hydroxy-4-methylanthra-9, 10-quinone	$C_{15}H_{10}O_3$	238	
11	30.35	4-(2-Oxiranyl)-9H-fluoren-9-ol	$C_{15}H_{12}O_2$	224	
12	33.14	6,9-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	

Results and Discussion

Auotodock

Auotodock vina was used to find out binding free energy (ΔG) (Nguyen *et al.*, 2019), between the ligand and protein docking complexes. Using binding free energy (ΔG), we can reveal the ligand molecules' binding affinity against target antimicrobial protein receptors. Binding free energy (ΔG) of all the ligand molecules was analyzed, and found that different ligands exhibit different binding affinity against antimicrobial proteins listed in Table 1. We considered the

docking complex with the lowest binding free energy (ΔG) for further interaction analysis among different docking conformations. We have analyzed the binding interactions and affinity of all docked complex molecules for detailed understating.

Molecular docking against *Xanthomonas oryzae* pv. *oryzae* (3H2G)

Among all ligand molecules, docking studies revealed that ligand 8 and ligand 10 showed the lowest binding free energy against Esterase LipA is -7.7 and -7.5 kcal/mol, respectively. These docking results showed that among the mentioned ligands, only Ligand 8 and 10 exhibited higher binding affinity. Different representations of the docking complex of ligand 8 and 10 with Esterase LipA shown in figure 1a-d and 2a-d. As shown in figure 1a-d of Ligand 8-receptor and figure2a-d of Ligand 10-Receptor docking complex, Pro87, THR 88, Ser176, Phe230, Leu233, Leu234, Leu275, Thr276, Phe279, Thr338, and His377 are common amino acid residues of Esterase LipA present at the docking site which involved interactions with both ligand molecules. Further, Tyr138, TYR175, Val339, Phe375, and Met378 residues interact only with ligand 8 but are not found in ligand 10. However, figure 1d show, only Tyr 175 interacts with ligand 8 by forming hydrogen bonds among other amino acids. Amino acid residues Gln177, His 180, and Met278 are found only in ligand 10- Esterase LipA docking complex, as shown in figure 2d. However, no hydrogen bond formation was found in the ligand 10- Esterase LipA docking complex.

Molecular docking against *Xanthomonas oryzae* pv. *oryzae* (3R5F)

Docking studies of ligands against the antimicrobial protein, D-alanine-D-alanine ligase a, results found that the lowest binding free energy of ligands molecules, ligand 8 and ligand 10, showed -6.8 and -6.0 kcal/mol respectively, among other ligand molecules.

These docking results depicted ligand 8 shows the highest binding affinity followed by ligand 10 against D-alanine-D-alanine ligase A. Different representations of the docking complex of both ligand 8 and ligand 10 against D-alanine-D-alanine ligase A shown in figure 3a-d and Figure4a-d. Analyzing the docking figures revealed several amino acids at the docking site to interact with ligand molecules. As shown in figure 3a-d, we found that Lys185, Val195, and Asn314 amino acids are found commonly on the docking site of D-alanine-D-alanine ligase A with both ligand 8 and 10. Further, as shown in Figure 3d and 4d, Ala156, Phe183, Glu221, Ala222, Ala223, Val224, Ala225, Glu228, Val249, and Phe304 are present only in the docking site of the Ligand 8-receptor complex. Among these amino acids, we found that Glu228 formed a Hydrogen bond and interacted with the ligand 8. In the docking complex, Ligand 10-D-alanine-D-alanine ligase A, we found lys141, Pro186, Gln189, Ser191, Ser192, Arg300, Glu315, and Asn317. Amino acids are present at the docking site, as shown in Figure 4d. It is found that Gln189 is the only amino acid that interacts with the receptor through the formation of a Hydrogen bond (Table 2).

These results collectively imply a strong binding affinity of ligand 8 and ligand 10 against both target proteins suggest these both ligands can block the activity of Esterase LipA proteins D-alanine-D-alanine ligase A. However, studies were carried out to check for their inhibitory effect against *Xanthomonas oryzae* pv. *oryzae*, which is mentioned in our previously published data Shobha *et al*. (2020) [12]. Our results co-related with that of Siddiquee et al. (2012) [14] where similar compounds were present, extracted from *Trichoderma harzianum*, identified by GC-MS analysis.

The presence of similar compounds may have contributed to the inhibition of Xoo and the enhancement of plant growth and yield. Therefore the Autodock was carried out in silico to know the binding affinity of the ligand molecule.

Table 2: Binding free energy (ΔG) of the ligand and Receptor docking complex using Autodock vina.

	Binding free energy (kcal/mol)	
Ligands	**Esterase Lip A (PDB ID-3H2G)**	**D-alanine-D-alanine ligase A (PDB ID-3R5F)**
1	-4.5	-4.9
2	-5.9	-4.6
3	-2.6	-2.6
4	-4.9	-5.3
5	-5.8	-4.6
6	-3.1	-3.1
7	-7.7	-6.8
8	-3.5	-3.0
9	-7.5	-6.0
10	-6.5	-5.5
11	-5.2	-5.3
12	-2.8	-2.7

Table 3: Docking of ligand and receptors involve amino acid interaction and H-bonds.

Ligand	Esterase Lip A (3H2G)	D-alanine-D-alanine ligase A (3R5F)
8	Pro87, THR 88, Tyr138, TYR175…H, Ser176, Phe230, Leu233, Leu234, Leu275, Thr276, Phe279, Thr 338, Val339, Phe375, His377, Met378.	Ala156, Phe183, Lys185, Val195, Glu221, Ala222, Ala223, Val224, Ala225, Glu228…H, Val249, Phe304, Asn314.
10	Pro87, Thr88, Ser176, Gln177, His 180, Phe230, Leu233, Leu234, Leu275, Thr276, Met278, Phe279, Thr338, His377.	lys141, Lys185, Pro186, Gln189…H, Ser191, Ser192, Val195, Arg300, Asn314, Glu315, Asn317.

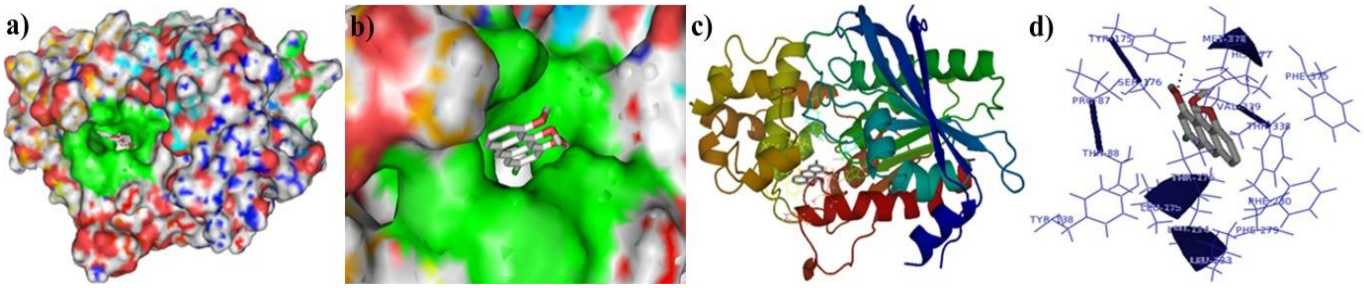


Fig 1: Schematic representation of Ligand 8 and Esterase LipA docking complex. (a) Overview of the surface model of ligand 8- Esterase LipA complex. b). Closer view of Ligand8-Esterase LipA docking complex. c) Ribbon model show ligand8 present at the docking site on the Esterase LipA. d) Ligand 8 interacts through amino acid residues of the Esterase Lip A.

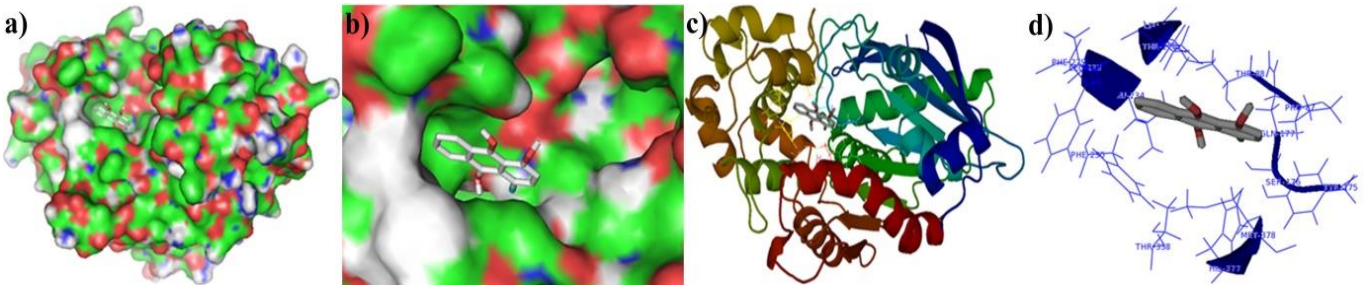


Fig 2: Schematic representation of Ligand 10 and Esterase Lip A docking complex. (a) Overview of the surface model of Ligand 10-Esterase LipA complex. b). Closer view of Ligand 10- Esterase Lip A docking complex. c) Ribbon model show ligand 10 present at the docking site on the Esterase Lip A. d) Ligand interact with protein through amino acid residues of the of Ligand 10-Esterase Lip A

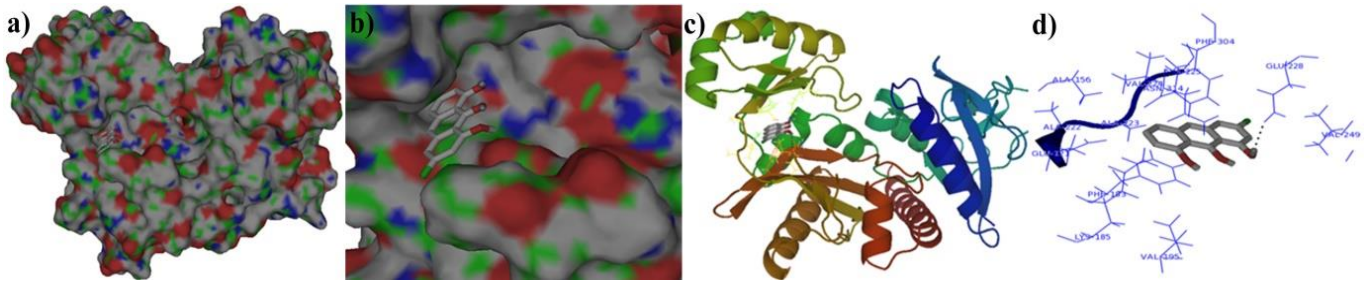


Fig 3: Schematic representation of Ligand 8 and D-alanine-D-alanine ligase A docking complex. (a) Overview of the surface model of of Ligand 8-D-alanine-D-alanine ligase a complex. b). Closer view of Ligand 8-D-alanine-D-alanine ligase A docking complex. c) Ribbon model show Ligand 8 present at the docking site on the D-alanine-D-alanine ligase A. d) Ligand 8 interact through amino acid residues of the D-alanine-D-alanine ligase A.

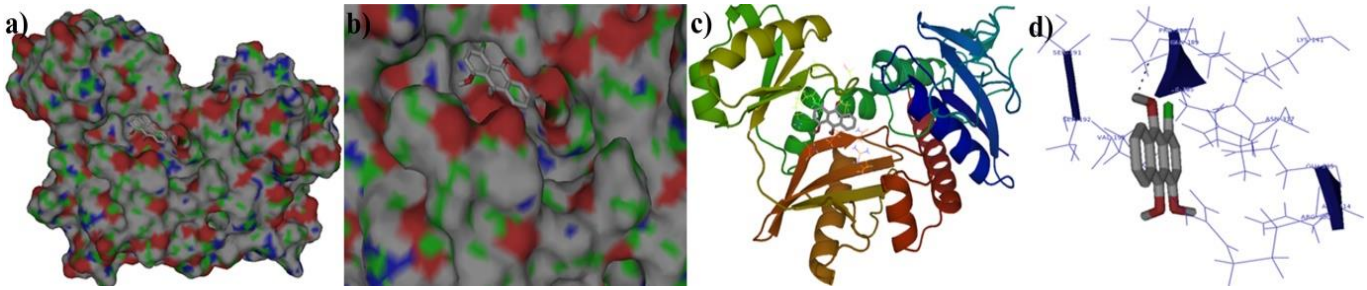


Fig 4: Schematic representation of Ligand 10 and D-alanine-D-alanine ligase a docking complex. (a) Overview of the surface model of Ligand 10-D-alanine-D-alanine ligase a complex. b). Closer view of Ligand 10-D-alanine-D-alanine ligase A docking complex. c) Ribbon model show ligand10 present at the docking site on the D-alanine-D-alanine ligase A. d) Ligand interact protein through amino acid residues of the Ligand 10-D-alanine-D-alanine ligase A.

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Conclusion
Rice production has become essential in order to fulfill the rice demand for the growing population. The synthesis of chemical bio fertilizers consumes enormous amounts of energy, contributing heavily to climate change. Their application in field has shown that they have adverse effects on human health and the environment. Therefore, environmental and consumer concerns have focused interest on the development of biological control. It is significant in being an ecologically conscious, cost-effective, and alternative strategy for chemicals. These organisms could be used as an alternative for bacterial leaf blight disease

management and plant growth promotion and enhance the yield. Docking results implied a strong binding affinity of the ligands against the target proteins suggests that these ligands can block the activity of proteins of Xoo.

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