In Silico Screening of Phytogenic Compounds Against Rhizoctonia Solani Trehalase Enzyme

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Short Report

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Abstract

The trehalase enzyme of *Rhizoctonia solani* is the target site for validamycin, a common fungicide used for controlling the sheath blight disease of rice. However, rampant use of validamycin has resulted in emergence of fungicide resistance necessitating the search for newer fungicide molecules. Thus, molecular docking analysis was carried out to screen phytogenic compounds with high trehalase inhibitory effect. The 3-dimensional structure of the protein was generated by the Swissmodel using the sequence information available in UniProt database (entry no. L8WUM1). Eighteen compounds from plants previously reported to have antagonistic effect against *R. solani* were selected for the study. Molecular docking carried out by Autodock 4.2 showed that the compounds, Cycloartenol (-8.64), β-Sitosterol (-8.58), Nimbiol (-8.29), Nimbandiol (-7.32), Menthone (-7.31), Nimbin (-7.22) had high binding energies whereas validamycin had a binding energy of -4.08. Among these, Nimbiol, Nimbandiol, Menthone, Nimbin were obeying all the Tice rule criteria and appeared to be good fungicide candidates against *R. solani.*

Introduction

Rice is the staple food for about one-fifth of world population [1]. The ever increasing population requires increased rice production. However, the rice diseases are among the major stumbling blocks for increased production. Among the diseases, the sheath blight disease caused by *Rhizoctonia solani* has been causing considerable yield loss which may go upto 50% [2, 3].

The disease is mostly controlled by application fungicides like validamycin, propiconazole, carbendazim etc. [4]. However, because of rampant use of these chemicals, chances of development of resistance of pathogens towards them has increased. In addition, there are environmental concerns associated with the use of synthetic pesticides. Therefore, efforts are being made to search biogenic compounds that could be used for managing plant diseases and pests.

Molecular docking has emerged as a useful tool for screening compounds with drug/pesticidal potential. This tool considerably minimizes the time required for searching drug/pesticide candidates [5]. In this context, molecular docking analysis was carried out to screen phytogenic compounds against the trehalase enzyme of *Rhizoctonia solani*. The trehalase enzyme in fungi is responsible the degradation of trehalose, which in turn act as a reactive oxygen species (ROS) scavenger [6]. It is also the target molecule for antifungal agents like validamycin and thus was selected as the target molecule in the present study.

Materials And Methods

Protein structure generation and validation

The protein structure for the trehalase enzyme of *Rhizoctonia solani* is not available in the protein data bank. Therefore, the amino acid sequence for the protein was retrieved from the SwissProt database in FASTA format. The 3-dimensional structure of the protein was generated using Swiss-model online server (https://swissmodel.expasy.org/). “Automodel” option was selected for the model generation which used neutral trehalase of *Saccharomyces cerevisiae* as template (sequence identity of 52.89% and a Global Model Quality Estimation (GMQE) value of 0.43). The PROCHECK Ramachandran plot was used for model validation.

Physico-chemical properties analysis, binding site prediction

The protein model generated by Swiss-model server was saved in .pdb format and it was used for physico-chemical property analysis using Protparam online (https://web.expasy.org/cgi-bin/protparam/protparam). The CastP server (http://sts.bioe.uic.edu/castp/index.html?1bxw) was used for determining the binding pockets for the modeled protein. The largest positive patch in the protein was determined using PatchFinderPlus server (http://pfp.technion.ac.il/).

Compound selection

Extracts from plants *Withania somnifera*, *Ziziphus jujube, Azardirachta indica, Mentha piperita, Ocimum basilicum, Eucalyptus, Juniperus polycarpus, Juniperus Sabina* antifungal activities against *Rhizoctonia solani*. Compounds like Rutin, kaempferol (*W. somnifera*), β-Sitosterol, stigmasterol, d-5-avenasterol, squalene, cycloartenol (*Z. jujube*), Nimbiol, nimbin, gedunin, nimbandiol, nimbolide (*A. indica*), Menthol, menthone, limonene (*Mentha piperita*), Linalool, eugenol (*Ocimum basilicum*), a-Pinene, limonene (Eucalyptus), Pinene (*J. polycarpus*, *J. sabina*) are among the principal constituents of these plants and were selected for the study [7–11]. Along with that validamycin which is known inhibitor of trehalase was included in the study. Propiconazole, another fungicide recommended for controlling the disease was also included in the study. Three dimensional structures of these compounds in .sdf format were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and were converted to pdbqt format using Openbabel software for docking.

Molecular docking

Molecular docking was performed using Autodock 4.2 software following methodology published earlier [12]. Briefly, Auto-grid program was used to generate the grid maps. The grid dimensions were 90 A° 90A° 90 A° with points separated by 0.375 A°. The grid was centered at -32.584, -0.973, -5.096 to encompass entire active site.

Pesticide likeliness test

The pesticide likeliness was computed only for the compounds which had high binding energy using the online tool available at http://www.molinspiration.com [12].

Results And Discussion

Protein structure generation and validation

The sequence retrieved from the SwissProt database was found to be having 774 amino. The 3-dimensional structure of the protein was generated using the Swiss-model tool (Fig. 1a) and it was validated using Ramachandran plot. It showed that 86.5% of the amino acid lied in the most favored region, additionally 11.5% was in the allowed region suggesting the validity of the model. The Ramchandran plot for the modelled protein is presented in Fig. 2.

Physico-chemical properties analysis, binding site prediction

ProtParam online server was used for determining the physico-chemical properties of protein. The molecular weight of the protein was calculated to be 88550.10 Da and the theoretical pI was calculated to be 5.73. The estimated half-lives of the protein in different organisms are as follows; 30 hours (mammalian reticulocytes, in vitro), > 20 hours (yeast, in vivo), > 10 hours (*Escherichia coli*, in vivo) which indicate the stability of the protein. The CastP program predicted the presence of five pockets with area > 100. However, the largest pocket was found to be having an area of 3586.440 and volume 3323.21. This binding pocket which fell in between the amino acids ILE-134 and TRP-655 was used for the grid preparation (Fig. 1b).

The PatchFinderPlus tool predicted the largest positive patch in the protein (Fig. 2) which contained the amino acids as follows: VAL104 ARG120 GLN90 ALA95 LEU94 LYS91 PHE614 MET121 ARG109 ILE103 HIS98 ILE648 ARG117 ARG100 (Fig. 1c).

Molecular docking

Molecular docking analysis showed the binding energies of the compounds. Table 1 presents the binding energies of different compounds along with the number of hydrogen bonds formed and the amino acids involved in it. Among the phytogenic compounds, Cycloartenol (-8.64), β-Sitosterol (-8.58), Nimbiol (-8.29), Nimbandiol (-7.32), Menthone (-7.31), Nimbin (-7.22), Nimbolide (-7.16) were found to be having high binding affinity. Validamycin which is a known trehalase inhibitor had a binding energy of -4.08. Similarly, propiconazole which is recommended as a fungicide to control the disease was found to be having a binding energy of -5.44. This suggest that the afore mentioned compounds have higher affinity for the trehalase enzyme compared to validamycin.

Table 1
The binding energies of different phytogenic compound as determined by Autodock 4.2 tool.

S.I. No.	Compound	Binding energy	No. of hydrogen bonds
1	Cycloartenol	-8.64	1 (GLU-237)
2	Beta-sitosterol	-8.58	-
3	Nimbiol	-8.29	-
4	Nimbandiol	-7.32	3 (ARG-234, LYS-624, LYS-147)
5	Menthone	-7.31	2 (PHE-284, LEU-285)
6	Nimbin	-7.22	3 (LYS 147, PRO-146)
7	Squalene	-7.15	-
8	Nimbolide	-7.16	1 (ARG-234)
9	Menthol	-6.97	1 (PRO-282)
10	pinene	-6.88	-
11	Limonene	-6.77	-
12	Gedunin	-5.92	1 (LYS-147)
13	Delta-5-avenosterol	-5.82	2 (GLU-237, ASN-236)
14	Stigmasterol	-5.82	1 (GLU-237)
15	Propiconazole	-5.44	-
16	Linallol	-5.65	1 (PHE-284)
17	Kaempferol	-5.31	3 (LEU-238, ASN-240, LEU-207)
18	Rutin	-5.3	5 (LYS-147, GLU-350, PRO-146, TYR-239, GLU-237)
19	Eugenol	-4.89	1 (PHE 284)
20	Validamycin	-4.08	5 (GLU-269, LEU-238, ASN-274, GLY-205, GLU-269)

Table 2
Molecular properties of compounds with low binding energy for determination of pesticidal suitability

	Molecular weight (kDa)	Hydrogen-bond donor (OH + NH)	Hydrogen-bond acceptor (O + N)	LogP (logarithm of the octanol/ water partition coefficient)	No. of rotatable bonds	No. of tice rule violations
Cycloartenol	426.73	1	1	8.21	4	1
Beta-sitosterol	414.72	1	1	8.62	6	1
Nimbiol	272.39	1	2	4.92	0	0
Nimbandiol	456.54	2	7	2.17	7	0
Menthone	154.25	0	1	3.15	1	0
Nimbin	488.96	0	9	3.55	8	0

Pesticide potency

The pesticide potency of the compounds with high binding energy was evaluated by the online server described in materials and methods section. The server works on the principle of Tice rule according to which, the chemicals to be used as pesticide should have the following properties; molecular weight ≤ 500 g/mol, no. of hydrogen bond donors (OH + NH) ≤ 3, no. of hydrogen-bond acceptors ≤ 12 (O + N), logP (logarithm of octanol/water partition coefficient) ≤ 5 and the no. of rotatable bonds ≤ 12 [14]. The compounds Cycloartenol and β-Sitosterol which were having the highest binding energies were found to be having one violation to the Tice rule (logP > 5). The other compounds with high binding energies like Nimbiol, Menthone, Nimbin, Nimbolide were meeting all the criteria set by the Tice rule.

Potential pesticide candidate

The binding energies of Cycloartenol, β-Sitosterol, Nimbiol, Menthone, Nimbin, Nimbolide were found to be much higher than validamycin which is a known inhibitor of the trehalase enzyme of *Rhizoctonia solani*. Out of these compounds, Nimbiol, Menthone, Nimbin, Nimbolide also met all the criteria set by Tice rule and thus appears to be better candidates to control the sheath blight disease of rice.

The compounds Nimbiol, Nimbin, Nimbolide are active compounds present in extracts of neem plant (*Azardirachta indica*) whereas menthone is found in *Mentha piperita.* These plants are readily available in the tropic to subtropical countries and could be used for extraction of the compounds.

This preliminary investigation identified compounds which have the potential to inhibit the growth of *R. solani* the causal agent of sheath blight disease. However, further *in vivo* studies needs to be carried out to see if these compounds can really control the disease.

Declarations

Acknowledgement:

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Ethics:

The study did not include any human or animal subjects and hence did not require any ethical approval.

Consent to Participate:

Not applicable.

Consent for Publication:

All authors read and approved the manuscript for publication.

Authors Contribution:

Conceptualization: AM, TAData curation: AM, TA; Formal analysis: AM, LB, SRP, SR; Investigation:AM, TA, LB, SRP, SR; Methodology:AM, TA, SL; Project administration: TA, SL, PCR; Writing ± review & editing: AM, TA, SL, PCR

Funding:

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Competing Interests:

None

Availability of data and materials:

All data are included in the article.

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Figures

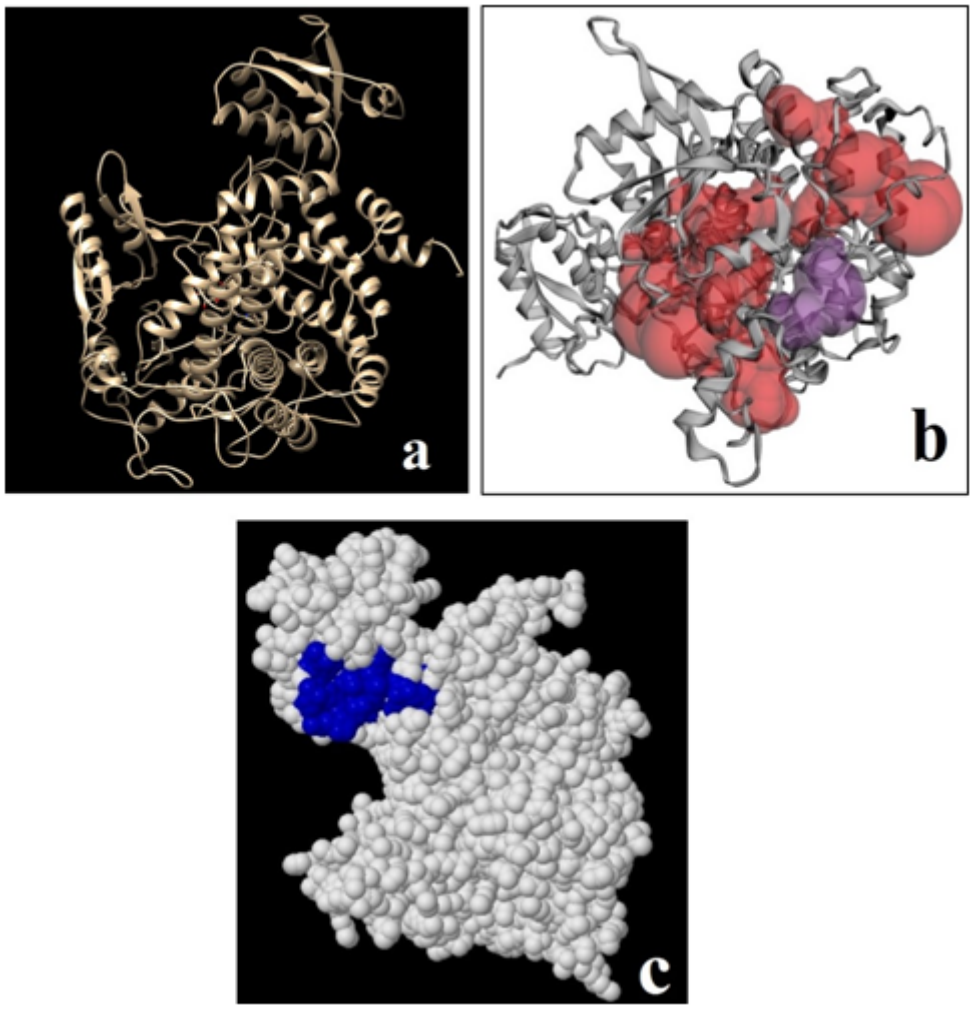

Figure 1

a. Three dimensional structure of trehalase enzyme of *R. solani* as visualized in Chimera software b. the binding pockets of the protein predicted by CastP online server c. the largest positive electrostatic patches on the protein surface as determined by the PatchFinder Plus.

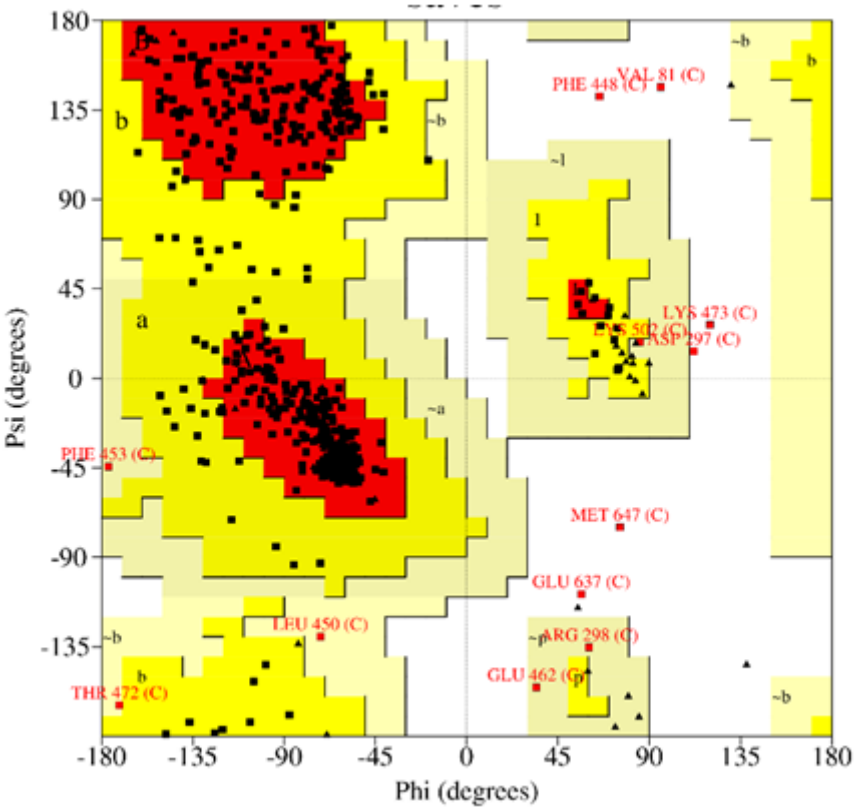

Figure 2

Ramchandran plot of modelled trehalase protein of *Rhizoctoniasolani*