In silico screening of phytogenic compounds against *Rhizoctonia solani* trehalase enzyme

Arabinda Mahanty¹ · Srikanta Lenka¹ · Totan Adak¹ · Lopamudra Behera¹,² · S. R. Prabhukarthikeyan¹ · S. Raghu¹ · Prakash Chandra Rath¹

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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 Swiss-model 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*.

**Keywords** In silico screening · Phytogenic compounds · *Rhizoctonia solani* · Sheath blight disease · Rice

**Introduction**

Rice is the staple food for about one-fifth of world population (FAOSTAT 2017). 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 up to 50% (Yugander et al. 2015; DRR 2017).

The disease is mostly controlled by application of fungicides like validamycin, propiconazole, carbendazim, etc. (Molla et al. 2020). However, because of rampant use of these chemicals, chances of development of resistance of pathogens towards them have 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 (Kurbanova et al. 2022). 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 for the degradation of trehalose, which in turn acts as a reactive oxygen species (ROS) scavenger (Wang et al. 2018). It is also the target molecule for antifungal agents like validamycin and thus was selected as the target molecule in the present study.

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¹ Crop Protection Division, ICAR-National Rice Research Institute, Bidyadharpur, Cuttack 753006, India
² Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, India
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 like *Withania somnifera*, *Ziziphus jujuba*, *Azadirachta indica*, *Mentha piperita*, *Ocimum basilicum*, *Eucalyptus*, *Juniperus polycarpus*, *Juniperus sabina* have been found to have antifungal activities against *Rhizoctonia solani* (EL-Hefny et al. 2020; Kagale et al. 2011; Ali et al. 2017; Abd-El-Khair and El-Gamal Nadia 2011; Khani et al. 2017). It was hypothesized that some of the compounds present in these plant extracts could be responsible for the antifungal activity of the extracts. Thus, the principal constituents of the plant extracts were considered for the study. Compounds like Rutin, Kaempferol are constituents of *W. somnifera*; β-Sitosterol, Stigmasterol, δ-5-avenasterol, Squalene, Cycloartenol are constituents of *Z. jujuba*; Nimbol, Nimbin, Gedunin, Nimbadiol, Nimbolide are constituents of *A. indica*; Menthol, Menthone, Limonene are found in *Mentha piperita*; Linalool, Eugenol are present in *Ocimum basilicum*, and a-Pinene is among the principal constituents of *Eucalyptus, J. polycarpus*, and *J. sabina*. Along with these compounds, Validamycin which is a 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 (Mahanty et al. 2021; Sidhu et al. 2020; Kumar et al. 2021). Briefly, Auto-grid program was used to generate the grid maps. The grid dimensions were 90 Å with points separated by 0.375 Å. 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 (Mahanty et al. 2021; Yadav et al. 2014).

Results and discussion

Protein structure generation and validation

The sequence retrieved from the SwissProt database was found to be having 774 amino acids. 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 Ramachandran plot for the modeled 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 the protein. The molecular weight of the protein was calculated to be 88,550.10 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 h (mammalian reticulocytes, in vitro), > 20 h (yeast, in vivo), > 10 h (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 (− 7.32), Nimbandiol (− 7.31), Menthone (− 7.22), 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 shows that the afore-mentioned compounds have higher affinity for the trehalase enzyme compared to validamycin.

**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 pesticides 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 number of rotatable bonds ≤ 12 (Tice 2001). 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, and Nimbolide were meeting all the criteria set by the Tice rule (Table 2) (Tice 2001).
Fig. 2 Ramachandran plot of modelled trehalase protein of *Rhizoctonia solani*

**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      | Linalool               | −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) |
Potential pesticide candidate

Trehalose is a disaccharide found in fungi and insects which is synthesized under stress condition. When the cells return to normal state, trehalose gets degraded by the trehalase enzyme into two glucose molecules. Thus, the dynamics of trehalose and trehalase play an important role in the physiology of the fungus and trehalose inhibitors like Validamycin are used for controlling the fungal diseases. Validamycin works as a competitive or non-competitive inhibitor of trehalase (García and Argüelles 2021; Wang et al. 2022). Although, the exact binding site of trehalase to which Validamycin attaches has not been structurally elucidated, the present study showed that the amino acids GLU-269, LEU-238, ASN-274, GLY-205, and GLU-269 are involved in the H-bonding. In the present study, a number of compounds like Cycloartenol, Beta-sitosterol, Nimbiol, Nimbandiol, Menthone, and Nimbin had higher binding energies than Validamycin indicating that like Validamycin, these could also inhibit the activity of trehalase. Out of these compounds, Nimbiol, Menthone, Nimbin, and Nimbolide also met all the criteria set by Tice rule and thus appear to be better candidates to control the sheath blight disease of rice. The compounds Nimbiol, Nimbin, and Nimbolide are active compounds present in extracts of neem plant (*Azadirachta 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 which could lead to production of economical and environment friendly fungicides for controlling the sheath blight disease of rice.

This preliminary investigation identified the compounds which have the potential to inhibit the growth of *R. solani* the causal agent of sheath blight disease. However, in vitro antifungal assays such as the “poisoned food method” needs to be employed for validating the findings of the present study (Adak et al. 2020; Thankaraj et al. 2022). Further, in vivo studies in pot cultures and field condition need to be carried out to see if these compounds can really control the disease. Moreover, the mechanism through which these compounds could be inhibiting the activity of trehalase enzyme needs to be investigated.

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Availability of data and materials All data are included in the article.

Declarations

Conflict of interests The authors declare that they have no competing interest.

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.

References

Abd-El-Khair H, El-Gamal Nadia G (2011) Effects of aqueous extracts of some plant species against *Fusarium solani* and *Rhizoctonia solani* in *Phaseolus vulgaris* plants. Arch Phytopath Plant Prot 44(1):1–16

Adak T, Swain H, Munda S, Mukherjee AK, Yadav MK, Aravindan S, Bag MK, Rath PC (2020) Green silver nano-particles: synthesis using rice leaf extract, characterization, efficacy, and non-target effects. Environ Sci Pollut Res 28:4452–4462

Ali EOM, Shakil NA, Rana VS, Sarkar DJ, Majumder S, Kausik P, Singh BB, Kumar J (2017) Antifungal activity of nano emulsions of neem and citronella oils against phytopathogenic
fungi, *Rhizoctonia solani* and *Sclerotium rolfsii*. Ind Crops Prod 108:379–387

DRR (2017) Production oriented survey. In: Annual progress reports, All India Coordinated Rice Improvement Projects (AICRIP), Hyderabad, India

El-Hefny M, Salem MZM, Behiry SI, Ali HM (2020) The potential antibacterial and antifungal activities of wood treated with *Withania somnifera* fruit extract, and the phenolic, caffeine, and flavonoid composition of the extract according to HPLC. Processes 8(113):1–13

FAOSTAT (2017) Crops/Regions/World list/Production Quantity (pick lists), Rice (paddy), 2018. UN Food and Agriculture Organization, Corporate Statistical Database 2020. Archived from the original on May 11, 2017

García MD, Argüelles JC (2021) Trehalase inhibition by validamycin A may be a promising target to design new fungicides and insecticides. Pest Manag Sci 77:3832–3835

Kagale S, Marimuthu T, Kagale J, Thayumanavan B, Samiyappan R (2011) Induction of systemic resistance in rice by leaf extracts of *Zizyphus jujuba* and *Ipomoea carnea* against *Rhizoctonia solani*. Plant Signal Behav 6(7):919–923

Khani A, Rashid B, Mirshekar A (2017) Chemical composition and insecticidal efficacy of *Juniperus polycarpus* and *Juniperus sabina* essential oils against *Rhizoctonia solani*. Int J Food Prop 20(S2):S1221–S1229

Kumar RM, Anantapur R, Peter A (2021) Unravelling the natural dual-target inhibiting potential of cucurbit bioactive compounds for the management of cucumber mosaic virus (CMV) through computational approaches. J Protein Proteom 12:307–324

Kurbanova M, Saravanak R, Ahmad S, Sadigova A, Askerov R, Mageramov A, Bakri YE (2022) Computational binding analysis of ethyl 3,3,5,5-tetracyano-2-hydroxy-2-methyl-4,6-diphenylcyclohexane-1-carboxylate in Calf Thymus DNA. Appl Biochem Biotechnol. https://doi.org/10.1007/s12010-022-03849-0

Mahanty A, Lenka S, Rath PC, Raghu S, Prabhukarthikeyan SR (2021) In silico docking of natural compounds from plants against *Rhizoctonia solani* pectate lyase. J Protein Proteom 12:63–69

Molla KA, Karmakar S, Molla J, Bajaj P, Varshney RK, Datta SK, Datta K (2020) Understanding sheath blight resistance in rice: the road behind and the road ahead. Plant Biotechnol J. https://doi.org/10.1111/pbi.13312

Sidhu KS, Bhanuk SK, Pathak RK, Yadav IS, Chhuneja P (2020) Identification of natural lead compounds for leaf rust of Wheat: a molecular docking and simulation study. J Protein Proteom 11:283–295

Thankaraj SR, Kumaradhass HG, Iyadurai AP, Arjunan M, Hudson AS, Chelladurai J (2022) Proteomic approaches to identify resistance proteins from *Rhizoctonia solani* infected rice, treated with seaweed and bioinoculants. Indian Phytopathol 75:477–486

Tice CM (2001) Selecting the right compounds for screening: does Lipinski’s Rule of 5 for pharmaceuticals apply to agrochemicals? Pest Manag Sci 57:3–16

Wang C, Pi L, Jiang S, Yang M, Shu C, Zhou E (2018) ROS and trehalose regulate sclerotial development in *Rhizoctonia solani* AG-1 IA. Fungal Biol 122:322–332

Wang C, Zhao M, Shu C, Zhou E (2022) Three genes related to trehalose metabolism affect sclerotial development of *Rhizoctonia solani* AG-1 IA, causal agent of rice sheath blight. Rice Sci 29(3):268–276

Yadav RP, Ibrahim KS, Gurusubramanian G, Kumar N (2014) In silico docking studies of non-azadirachtin limonoids against ecdysone receptor of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). Med Chem Res 24:2621–2631

Yugander A, Ladhalakshmi D, Prakasham V, Mangeaithia SK, Prasad MS, Krishnaveni D, Madhav MS, Sundaram RM, Laha GS (2015) Pathogenic and genetic variation among the isolates of *Rhizoctonia solani* (AG-IA), the rice sheath blight disease. J Phytopathol 163:465–474

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