

Insilico Analysis and Screening of Novel Target for Beta1, 3 Glucanase D Involved In Red Rot

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ABSTRACT

Sugarcane (*Saccharum officinarum*) is a vital cash crop and agro-industrial product, especially in India, which is one of the largest producers and consumers of sugar. However, its cultivation is severely affected by various diseases, with red rot—caused by the fungal pathogen *Colletotrichum falcatum*—being one of the most destructive. This disease, often referred to as the "cancer of sugarcane," leads to major losses in both yield and quality. The fungus is highly variable in nature, which frequently leads to the failure of resistant varieties due to the emergence of new, more virulent strains.

Red rot has been reported for over a century in many sugarcane-growing countries including India, Java, Argentina, and the USA. Despite efforts like hybridization between cultivated sugarcane and wild species to breed resistant varieties, periodic outbreaks have occurred due to the widespread cultivation of a single variety and the breakdown of resistance over time.

To understand the biology of *C. falcatum* and aid in disease management, this study focused on identifying and modeling potential drug target proteins from the pathogen. A protein sequences were retrieved from the UniProt database, and 3D structural models were developed using tools like Swiss Model and BLASTp. models were validated as significant using Ramachandran plot analysis.

This dataset of protein structures could be a valuable resource for targeted drug discovery and development of antifungal treatments for managing red rot disease in sugarcane. Additionally, recent genomic and transcriptomic studies on *C. falcatum* have identified potential effector genes and pathogenicity hotspots, which can support future efforts in genetic improvement and integrated disease management strategies. Other

approaches including bioagents, chemical treatments, and mechanized sett treatments are also showing promising results in controlling the disease in the field.

Keywords: Red rot, *Colletotrichum Falcatum*, Homology Modling, Ramachandran Plot, Red Rot of Sugarcane, Drug targets, Swiss-Model, Molecular Docking,

I. INTRODUCTION

Sugarcane (*Saccharum officinarum*) is one of the most important commercial crops globally. It is grown in over 100 countries and provides nearly 75% of the world's sugar. Apart from sugar production, sugarcane is also used in the production of ethanol (abiofuel), jaggery, molasses, rum, and other products such as paper and livestock feed through its by-product called bagasse. This makes sugarcane a vital crop not only for food industries but also for energy and industrial use. It is mainly cultivated in tropical and subtropical countries like Brazil, India, China, and Thailand (Dixon et al., 2018). Sugarcane is a rich source of essential minerals such as calcium, magnesium, potassium, sodium, and iron. Its juice contains saccharose and organic acids that contribute to its nutritional value. The plant belongs to the Poaceae family and the genus *Saccharum*, and is a tall, fibrous, jointed-stalk grass. It is estimated that sugarcane cultivation occupies more than 26 million hectares of land globally and supports millions of farmers (Sumit et al., 2014). Despite its importance, sugarcane production faces several challenges. Among them, diseases caused by fungi, bacteria, and viruses pose serious threats to yield and quality. One of the most damaging diseases is red rot, which has been known for more than a century and continues to be a major issue in sugarcane-producing countries.

Causal Organism: *Colletotrichum falcatum* Red rot disease is caused by a fungal pathogen called *Colletotrichum falcatum*. It belongs to the

Glomerellaceae family. This fungus infects the sugarcane stalks and causes reddish discoloration of the internal tissues, which later turn into dark brown or black areas. As the disease progresses, the infected stalks may become hollow, dry, and weak. This leads to poor juice quality, reduction in sugar content, and eventually the death of the plant (Viswanathan, 2021).



Figure:1 Cut ends of red rot- affected canes show reddish discoloration with white patches and fungal mycelium filled in the pith cavities.



Figure 2. Wilt-affected canes exhibit typical hollowness in the pith region with brownish discoloration in the rim of the pith cavities.

The pathogen spreads mainly through infected planting material, rainwater, and crop debris left in the field. Conditions such as continuous planting of the same sugarcane variety, high soil moisture, and poor field hygiene encourage the spread of the disease. The fungus produces spores (conidia) that enter the plant through wounds or natural openings and begin to grow inside the stalk (Souza et al., 2017).

Red rot is one of the most serious diseases of sugarcane in many countries including India, Pakistan, Bangladesh, Thailand, Myanmar, Nepal, Vietnam and other countries. In Louisiana, red rot was described as one of the factors causing stubble deterioration of sugarcane

Red rot is widespread and has been reported in more than 75 sugarcane-growing countries including India, Pakistan, Bangladesh, Thailand, Brazil, Fiji, and the USA. In India, the disease was first observed in 1901 and has since caused multiple epidemics, especially in the Gangetic plains and tropical regions. In several cases, entire commercial varieties had to be removed from cultivation due to disease outbreaks (Menossi et al., 2008).

Impact on Yield and Resistance Breakdown

Red rot severely affects sugarcane productivity. It can cause a reduction of 29–83% in cane weight and a loss of 31–75% in sugar content (Ahmad et al., 1986). Several elite varieties once thought to be resistant have failed over time due to the appearance of more virulent strains of *Colletotrichum falcatum*. This is mainly due to a phenomenon known as the 'vertifolia effect', where continuous planting of one variety over large areas allows the pathogen to adapt and overcome the plant's defenses. Efforts have been made to control red rot by breeding resistant varieties through interspecific hybridization between *S. officinarum* and wild relatives like *S. spontaneum*. This process, known as nobilization, helped introduce new resistant lines. However, even these varieties eventually become susceptible as new fungal strains evolve, making red rot a continuous threat (Viswanathan, 2021).



Figure.3: Distribution of sugarcane red rot in the world

(<https://www.cabi.org/isc/datasheet/25361#toDistributionMaps>)

II. MATERIALS AND METHODS

1. Retrieval of Protein Sequence

The protein sequence of beta-1,3- glucanases was retrieved by using UniProt protein database and saved in the FASTA file format. UniProt is the Universal Protein Resource is comprehensive resource for protein sequence and annotation data. The amino acid sequences accessible protein sequence database UniProt[10]

2. Physiochemical Properties Analysis

The secondary structure was predicted using. The ProtParam has been described as increasing the probability of proteins, the ProtParam method accurately predicts 70.00% of amino acids for a three. The physiochemical properties of Beta 1,3 glucanase D was analyzed by using Proparam the computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half- life, instability index, aliphatic index and grand average of hydropathicity (GRAVY)[11]

3. Secondary Structure prediction and analyses

successful protein secondary structure prediction. In a database of 126 chains of non-homologous (less than 25% identical) state description of

the secondary structure (alpha-helix, beta-sheet, and coil).[12]

4. Homology Modelling and Model Validation

Beta 1,3 glucanase D were used for comparativehomologymodellingbyusing SWISS MODEL server. An annotated database of fully automated homology- modellingpipelineSWISS-MODEL-generated three-dimensional comparative protein structure models is the SWISS- MODEL Repository. The Storehouse right now contains around 300,000 three- layered models for groupings from the Swiss-Prot and TrEMBL information bases. After modelling, we can check the quality and validation of the model was carried out by PROCHECK Ramchandran plot method using PDBsum server. [13]

III.RESULTSANDDISCUSSION

1. Retrieval of Beta1,3 glucanase D protein

The protein sequence of Beta 1,3glucanase D protein was retrieved from UniProt database and stored in the FASTA file in notepad. The protein name, organismname, UniProtKBID and sequence length is given below in the table no.1

Protein Name	Organism Name	UniProtKB ID	Seque nce Length
Beta 1,3 glucanase D	Saccharum officinarum	A0A1B3B2K9	74

Tableno.1Retrievalofsequence

2. Physiochemical analysis of protein

The physiochemical properties were analysed by using ProtParam tool and resultswereenlistedinthegiventable no.2

3. Secondarystructureprediction

helix, beta sheets, extended strands, and randomcoilsweregiven belowinthetable no.3.From the given table the percentageof coils of Beta 1,3

glucanase D is higher than percentage of alpha helix and extended strand.

Parameters	Values
Total No. of amino acid	70
Molecular Weight	7645.71
TheoreticalPi	5.81
UniProtKBID	A0A1B3B2K9
The no ofpositive amino acids (Arg+Lys)	5
The no of negative amino acids (Asp+Glu)	7
Aliphaticindex	100.14
GRAVY	0.076

Table no.2 Physiochemical parameters of β 1,3 glucanase D

The secondary structure of protein was predicted by using the GOR4 method. The secondarystructureelementslikealpha

Secondary Structure elements	Numberof Residues	Percentage
Alpha Helix	21	30.00%
Extended Strand	14	20.00%
BetaTurn	0	0.00%
Random Coil	35	50.00%

TableNo.3SecondaryStructurePrediction

4. Homology Modelling and Model Validation

The 3D structure of Beta 1,3 glucanase D was predicted by using SWISS Model server. The sequence template Beta 1,3 glucanase D protein were selected as template for prediction of homology modelling. The quality and validation of modelwascarriedoutbyRamchandran plot analysis using PDBsum server. Ramchandran plot analysis showed that percentage of favored region is 92.3.% which was higher than percentage of additional allowed region it concludes predicted model was reliable and goof quality shown below in the Fig no.1 Further model was visualized in the RasMol

visualization software packages given below in fig no 2

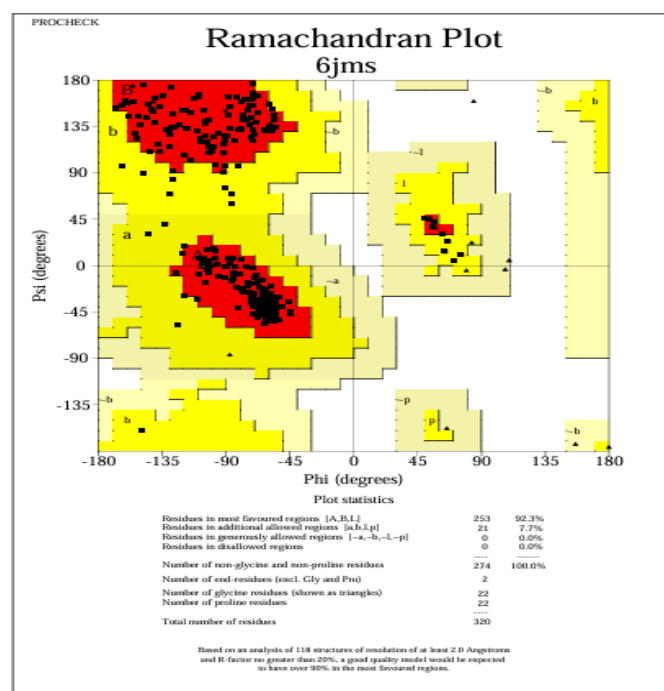


Figure.No.4 Protein Validation by PROCHECK

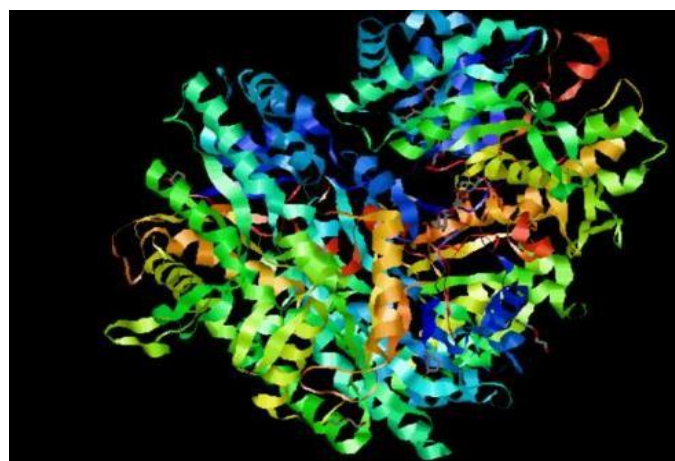


Figure.No.5 Visualization of Predicted 3D structure by RasMol

5. Identification of ADME properties and toxicity of drugs using SwissADME

The pharmacokinetic properties of 259 apigenin synthetic compounds were evaluated using SwissADME, applying Lipinski's Rule of Five, P-gp substrate prediction, BBB permeability, and other drug-likeness filters. In-house tools like the BOILED-Egg model, iLOGP, and Bioavailability Radar were also used for screening. From the analysis, six lead molecules, including apigenin, were shortlisted for further toxicity prediction. Among them, apigenin compounds that passed ADMET criteria were found to be non-carcinogenic and non-mutagenic, indicating their non-toxic nature.

6. Molecular docking and analysis of protein-ligand interaction-

Three synthetic and seven natural compounds passed Protox3.0 screening and were selected for docking against beta-1,3-glucanase (4hpg.1) protein. Docking showed that molecules 1 to 5 formed stable complexes with favorable binding energies and multiple hydrogen bonds. Among them, especially molecule 1 (3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one) showed strong binding affinity. These results suggest their potential as effective, non-toxic drug candidates.

Table 4: Results of synthetic compounds showing highest binding energy

Compound Name	Binding energy	H bonds	Intermol energy	Vdw_hb desolv energy	Electrostatic energy	Total internal
Catechin	-6.1	4	-12.4	-6.28	-0.11	-0.33
Kaemferol	-6.01	4	-6.31	-6.12	-0.19	0.3
Fenarimol	-5.56	1	-6.46	-6.46	0	0.59
Caffeic acid	-4.8	3	-5.39	-3.82	-1.57	0.1
Apigenin	-4.39	2	-4.69	-2	-0.09	0.24

Compound Name	Binding energy	H bonds	Intermol energy	Vdw_hb desolvenergy	Electrostatic energy	Total inernal
Benomyl	-3.87	2	-5.36	-5.31	-0.06	0.33
Carbendazim	-3.56	2	-4.16	-4.04	-0.11	.0.12

IV.CONCLUSION

The present preliminary investigation mainly leads to understand basic primary, secondary structure and tertiary structure of Beta 1,3glucanase D In-Silico Tools and Technique. The Beta 1,3glucanase D were retrieved from UniProt database and having length of 70. The primary sequence analysis carried out by using ProtParam tool and retrieval having Molecular weight of 7645.71Da, theoretical pI 5.81, the no of positive amino acids (Arg+Lys):5, the no of negative amino acids (Asp+Glu):7, respectively Aliphatic index:100.14, and GRAVY:-0.076.

Secondary structure of Beta 1,3glucanase D was predicted by using ProtParam having alpha helix 21, beta turn 0, extended strand 14, and random coil 35 so protein structure was highly stable. The Modelling of 3D structure of protein Beta 1,3glucanase D was build by using SWISS Model. Structure was validated using PROCHECK having model quality was 92.3%. This present study put molecular insight into the further studies to find structural and functional properties of Beta 1,3glucanase D

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