Genome mining of secondary metabolites from *Streptomyces* spp. for the screening of novel and potent fungicide: Focus on rice blast disease

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Abstract

Rice stands as the most consumed commodity globally, serving as the primary energy source for nearly half of the world's population. However, rice blast disease, caused by the fungus Magnaporthe oryzae, leads to significant production losses. This research focuses on devising a sustainable solution against M. oryzae using advanced computational techniques including genome mining and in silico screening. The melanin pathway in M. oryzae involving the conversion of tyrosine to melanin is crucial for its pathogenicity. Succinate dehydrogenase (SDH) plays a key role in this pathway by catalysing the oxidation of succinate to fumarate, thereby contributing to the production of melanin. Currently, carpropamid is used as a commercial inhibitor for rice blast disease due to its broad-spectrum fungicidal activity. However, its unintended harm to non-target organisms presents limitations, highlighting the need for alternative solutions to combat rice blast disease.

Streptomyces-derived bio-fungicides emerge as a promising alternative, harnessing bioactive chemicals produced by plant endophytic bacteria to suppress pathogen growth. The research leveraged genome mining from the 23 whole genome sequence of plant endophytic streptomyces. This process yielded 3343 putative molecules and further reduced to 906 compounds after removing duplicates. These compounds were subjected to a series of molecular simulation strategy to unveil the potent fungicide compound. Ultimately, the study identifies Cremeomycin and Azetidomonamide B as secondary metabolites with superior binding affinity, interaction and fungicidal potential compared to carpropamid. The future trajectory of this research entails experimental validation and the development of sustainable bio-fungicides against rice blast disease.

Keywords: *Magnaporthe oryzae*, Succinate dehydrogenase, *Streptomyces*, Cremeomycin and Azetidomonamide B.

Introduction

Rice (*Oryza sativa L.*) is a staple food crop and an energy source for half of the world's population. India, the second-largest producer globally, cultivates approximately 135 million metric tons of rice[9]. However, various biotic stressors such as pathogen invasion and insect infestations, along with abiotic stresses like extreme temperatures, heavy metal toxicity and salinity, render the crop into susceptible nature[25]. Among biotic stressors, rice blast disease caused by the fungus *Magnaporthe oryzae* is one of the most devastating diseases affecting rice production worldwide. The rice blast can lead to crop failure during various growth stages, with annual crop losses worldwide ranging from 10% to 30%[24]. India reports crop losses of 5% to 10%, especially in the humid regions of southern India. With the blast pathogen causing an alarming annual loss of $66 billion, it is crucial to protect the rice crop from the disease[10].

Understanding different target-specific inhibitors that regulate the pathogenesis of rice blasts will aid in developing plausible solutions to control pathogen infection. The pathogenesis of rice blast disease involves several intricate processes including spore germination, host recognition, penetration, colonization and symptom development. Initially, airborne spores called conidia land on rice plants and germinate in response to environmental factors like moisture and temperature[17]. This germination leads to the formation of infection structures called appressoria. The fungus detects chemical cues from the rice plant and facilitates appressoria growth. Appressoria exert mechanical pressure to penetrate the host's cuticle and cell wall, initiating the colonization of rice tissues.

Within the host, the fungus multiplies, extending hyphae, which leads to the water-soaked lesions that become necrotic and release spores, ultimately spreading the disease. Factors such as high relative humidity (above 80%), a higher dosage of nitrogen fertilizers, the presence of collateral hosts and sluggish wind provide a favourable environment to facilitate this process[28].

To overcome rice blast disease, several management techniques have been used such as host resistance, fungicide administration and biological control[16]. Fungicides like Pyroquilon, Carbendazim, Carpropamid and Tricyclazole are widely used in crop fields. Among them, carpropamid is a broad-spectrum fungicide used to control fungal diseases in crops. Its protective and curative properties can stop and prevent fungal growth when administered at the proper time. However, its fungicidal effectiveness is mainly offset by unintended harm to non-target organisms. Adding together, the rapid development of pathogen variants and their ability to overcome host resistance highlights the necessity for discovery of novel fungicidal agents[29].

In recent years, plant endophytic bacteria have offered various benefits in agriculture, such as promoting plant growth, improving stress tolerance and providing protection against biotic and abiotic stresses[12]. Endophytes possess the ability to boost plant immunity against pathogenesis-related proteins and the production of phytohormones to combat pathogen stress[5].

Specifically, endophytic *Streptomyces* species and their secondary metabolites contain a wide range of bioactive chemicals that can be employed as antifungal elements against *Magnaporthe oryzae*[20]. For example, the biosynthesis of antimicrobial metabolites such as ectoine, desferrioxamine B and geosmin by *Streptomyces albidoflavus* contributes to its antifungal properties in the rhizosphere of *Salvia miltiorrhiza*[6]. Bio-fungicides derived from *Streptomyces* present a viable substitute for chemical fungicides, with possible advantages including a diminished ecological footprint and susceptibility to resistance, making it a suitable candidate for further studies[7].

Thus, the present study aimed to leverage genome mining approach for screening of secondary metabolites from plant endophytic *Streptomyces* species to target *Magnaporthe oryzae*. Overall, we hope that this study will develop a sustainable solution to manage rice blast disease effectively.

Material and Methods

Genome mining: A systematic literature review was conducted for plant endophytic Streptomyces species and identified 23 complete *Streptomyces* spp. genomes (Table 1). To predict the secondary metabolites from the collected *Streptomyces* genomes AntiSMASH (Antibiotics and Secondary Metabolite Analysis Shell) version 7.0 webserver (https://antismash.secondarymetabolites.org/) was utilized[4]. The detection strictness was set to "strict," to detect well-defined clusters from the sequence.

Subsequently, the parameters such as KnownClusterBlast, ClusterBlast, SubClusterBlast, MIBiG (Minimum Information about a Biosynthetic Gene Cluster) cluster comparison and ActiveSiteFinder were enabled to effectively predict and compare gene clusters. Further, the compound carpropamid (PubChem ID: 449402), a commercial fungicide targeting *Magnaporthe oryzae*, was selected as the reference compound for comparative analysis.

Table 1
Details of *Streptomyces* species along with their NCBI Accession IDs

S.N.	NCBI Accession ID	Organisms
1	NZ_CP040466.1	*Streptomyces albidoflavus*
2	NZ_CP045095.1	*Streptomyces phaeolivaceus*
3	NZ_CP047020.1	*Streptomyces broussonetiae*
4	NZ_CP051486.1	*Streptomyces pratensis*
5	NZ_CP060825.1	*Streptomyces genisteinicus*
6	NZ_CP060828.1	*Streptomyces roseirectus*
7	NZ_CP061282.1	*Streptomyces xanthii*
8	NZ_CP102514.1	*Streptomyces yangpuensis*
9	NZ_CP104864.1	*Streptomyces lusitanus*
10	NZ_CP021748.1	*Streptomyces alboflavus*
11	NZ_CP063844.1	*Streptomyces sp. A10(2020)*
12	NZ_CP011522.1	*Streptomyces sp. CFMR 7*
13	NZ_CP101649.1	*Streptomyces sp. CRLD-Y-1*
14	NZ_CP090447.1	*Streptomyces sp. CRSS-Y-16*
15	NZ_CP110636.1	*Streptomyces sp. HNM0140*
16	NZ_CP066801.1	*Streptomyces sp. HSG2*
17	NZ_CP034353.1	*Streptomyces sp. KPB2*
18	NZ_CP043958.1	*Streptomyces sp. LBUM 1475*
19	NZ_CP043957.1	*Streptomyces sp. LBUM 1480*
20	NZ_CP043956.1	*Streptomyces sp. LBUM 1482*
21	NZ_CP096907.1	*Streptomyces sp. LRE541*
22	NZ_CP048397.1	*Streptomyces sp. S4.7*
23	NZ_CP039123.1	*Streptomyces sp. SS52*
24	NZ_CP074111.1	*Streptomyces sp. V17-9*
25	NZ_CP079114.1	*Streptomyces sp. WY228*

Hierarchical Virtual screening: Initially, structural similarity between the reference and retrieved secondary metabolites was assessed using the Tanimoto coefficient (Tc) technique with the aid of Jupyter Notebook - RDKit environment. The Tc, ranging from 0 (no resemblance) to 1 (identical), indicates the degree of similarity between sets. Based on literature evidence, a threshold of 0.3 was applied as a selection criterion to filter similar molecules[13].

Compounds meeting this threshold were then subjected to molecular docking using Autodock Vina (v1.1.2). Scytalone dehydratase (SDH), complexed with carpropamid, was chosen as the molecular target and retrieved from the Protein Data Bank (PDB: 2STD). Protein preparation involved removing water molecules, adding Kollman charges, polar hydrogen atoms and Gasteiger charges. Ligands were similarly prepared using Open Babel[15].

Additionally, fungicidal-likeness analysis was performed on the docked compounds to identify environmentally friendly fungicides. This analysis considered molecular descriptors including molecular weight (MW $\leq$ 400 Da), partition coefficient (LogP $\leq$ 3), hydrogen bond acceptors (HBA $\leq$ 6), hydrogen bond donors (HBD $\leq$ 2) and rotatable bonds (RB $\leq$ 9). Compounds meeting all five criteria were selected for further analysis, with calculations performed using the Mcule.com property calculator (https://mcule.com/apps/property-calculator/).

Rescoring strategies: Machine learning scoring functions (ML-SF) were applied to rescore molecules filtered by fungicidal likeness. KDEEP (https://playmolecule.org/Kdeep/), Gnina (https://github.com/gnina/gnina?tab=readme-ov-file) and RFscore were used to predict protein-ligand affinity. KDEEP captured complex molecular features for accurate binding predictions, Gnina assessed ligand-receptor conformations and RFscore evaluated diverse interactions[2,14]. Ligands showing higher binding affinity than the reference compound was selected for further analysis. Finally, the interaction analysis reveals the binding pattern of the fungicide with the target protein[23]. Lead protein-ligand PDB files were prepared using PyMOL (v3.0) and non-covalent interactions were analyzed via the PLIP web server (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/ index).

Results and Discussion

AntiSMASH result from genome mining: AntiSMASH 7.0 predicted the secondary metabolites from various *Streptomyces* species, mainly found in the gene clusters such as terpenes, NI-siderophores, non-ribosomal peptide synthetases (NRPS), type I polyketide synthetases (T1PKS), ectoine, lanthipeptide-class (i, iii, iv), butyrolactone and type III polyketide (T3PKS). Among these, NRPS played a pivotal role in the realm of biofungicides due to their ability to target essential components of fungal cells, thereby hindering growth and proliferation[18]. Figure 1 illustrates the distribution of the BGCs among different *Streptomyces* species. Among them, *Streptomyces sp. CFMR 7* contains the maximum number of BGCs (137 regions) followed by *Streptomyces roseirectus* (45 regions). *Streptomyces xanthii* possesses the fewest number of BGCs, harbouring only two regions.

Ultimately, genome mining of 23 *Streptomyces* strains led to the discovery of 3343 secondary metabolites. Among these, the majority of the metabolites were dominated by terpenes (577 compounds), NI-siderophores (396 compounds), NRPS (254 compounds) and T1PKS (246 compounds) gene cluster types (Figure 2). Moreover, compounds such as ectoine and melanin occurred in the majority of the species. Following the removal of duplicates, 906 distinct compounds were retrieved and investigated.

Similarity results from Tanimoto coefficient: The similarity analysis between the reference and the 906 ligand compounds was conducted by utilizing the RDKit search algorithm. The findings of this investigation ranged from 0.2 to 0.5. Compounds with above the Tc score of 0.3 were chosen for subsequent analysis. Consequently, 548 compounds meeting this criterion underwent molecular docking procedures to gain insight into the inhibitory activity.

Binding affinity analysis using molecular docking: The AutoDock algorithms played a crucial role in predicting binding affinity and providing essential insights into the interactions between ligands and receptors[1]. In the investigation, 548 compounds identified through Tanimoto coefficient analysis underwent molecular docking, employing a grid dimension of center_x = 29.41, center_y = 36.95, center_z = 22.57, size_x = 80, size_y = 80, size_z = 80, num_modes = 10 and energy_range = 4 for the docking process. The results revealed binding affinities ranging from -11 to -1.5 kcal/mol between the SDH target protein and ligands.

Further, 427 compounds exhibiting binding affinities surpassing that of the reference compound, carpropamid (-5.3 kcal/mol) was earmarked for the fungicidal analysis. A concurrent investigation by Antony et al[3] examined the SDH protein and secondary metabolites derived from rice endophytic *Streptomyces* for their antifungal abilities. This research revealed that carpropamid exhibited a binding affinity of -8.882 kcal/mol when bound to the SDH^{WT} of *Magnaporthe oryzae*, demonstrating higher binding affinity to the protein. The findings of this study corroborate our results, demonstrating consistency in the observed binding characteristics of carpropamid.

Fungicidal likeliness analysis of lead molecules: Following molecular docking, fungicidal analysis evaluates the compounds' efficiency in inhibiting the growth or viability of fungi[22]. 427 compounds exhibiting satisfactory binding affinity were subjected to fungicidal analysis utilizing Mcule.com. The fungicidal properties of

carpropamid (PubChem ID: 449402) indicate that its calculated molecular descriptors fall within desirable ranges for all parameters except for Log P. Among 427 compounds, 54 compounds met all five molecular descriptor criteria and were subsequently selected for further scrutiny. It is noteworthy that low molecular weight and fewer hydrogen bond donors are crucial factors primarily responsible for ensuring the water solubility of agrochemicals[31]. Table 2 displayed the fungicidal properties of the lead compounds.

ML-SF rescoring analysis: A significant aspect of machine learning scoring functions (ML-SF) lies in their capacity to accurately predict the binding affinity between molecules[21,27]. The ML-SF models, such as KDEEP, Gnina and RFscoring, were employed to revalidate the binding affinity of the 54 compounds. The reference compound, Carpropamid, was found to have an RF score of 5.95, a KDEEP score of -6.55 and a Gnina score of 4.35.

From the 54 compounds, 14 compounds exhibiting a binding affinity higher than the reference in all three ML-SF were selected as lead compounds (Table 3). Notably, Salinosporamide A exhibited significantly higher binding affinity in KDEEP (-10.43 kcal/mol), while its scores in RF scoring and gnina were on par with the reference compound. Furthermore, azetidomonamide B demonstrated binding affinity similar to the reference compound across all three scoring functions. A high ML-SF score for a compound indicates a strong predicted binding affinity with the target molecule, suggesting effective interaction. Table 4 outlines lead bioactive compounds from endophytes and their biosynthetic gene cluster types.

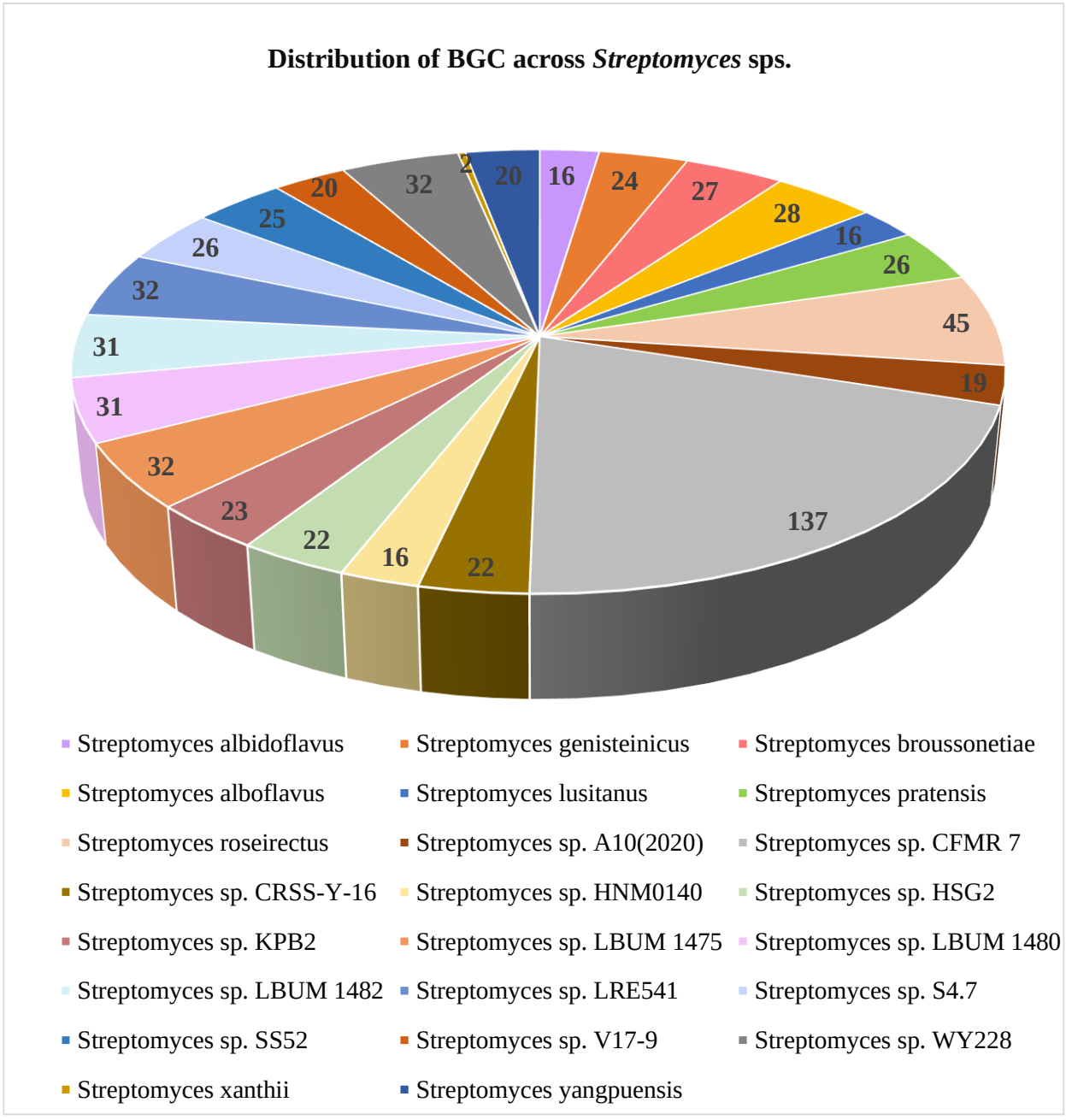

Figure 1: The distribution of biosynthetic gene clusters across 23 plant endophytic *Streptomyces* species

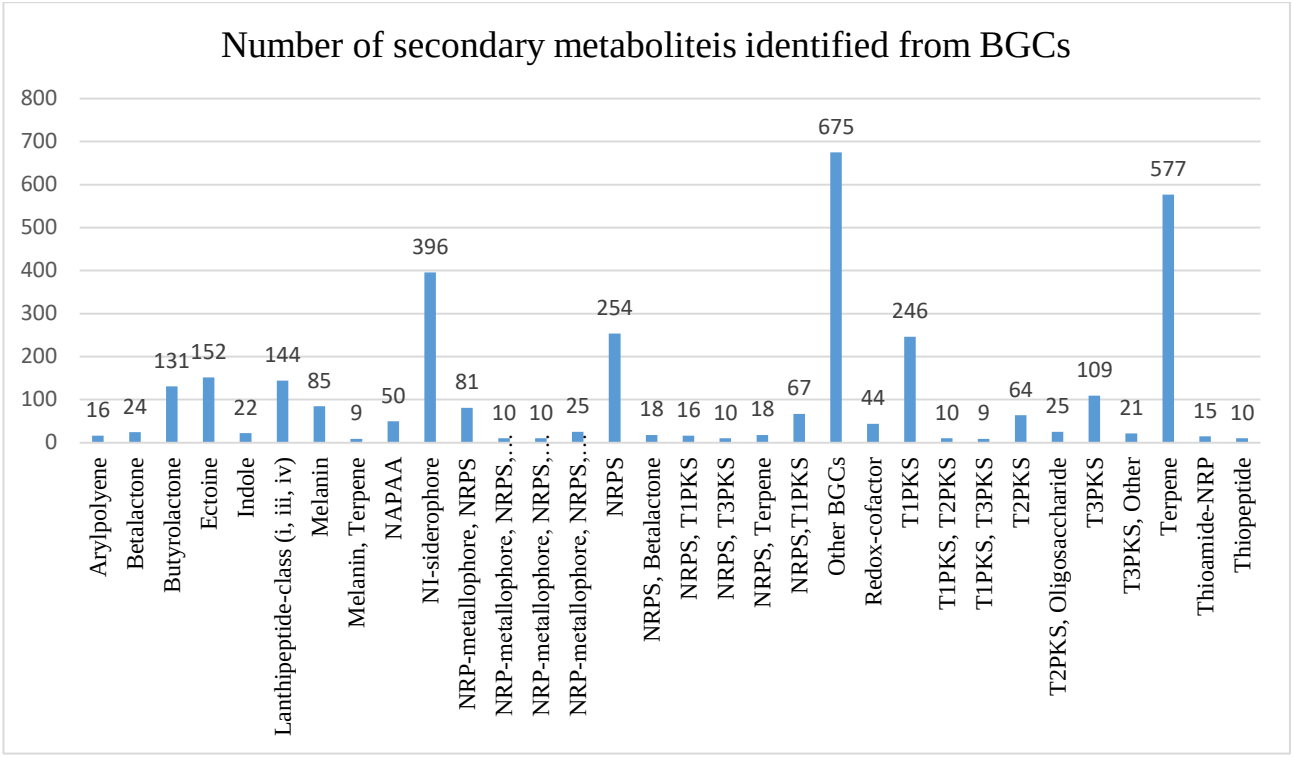

Figure 2: The number of secondary metabolites identified from biosynthetic gene clusters.

Table 2
Fungicidal analysis result of the lead compounds

S.N.	Compound	MW (<400) Da*	LogP (<3) *	HBA (<6) *	HBD (<2) *	RB (<9) *
1	Carpropamid (Reference)	334.66	5.13	2	1	5
2	Shanorellin	182.17	0.28	4	2	1
3	Mellein	178.18	1.49	3	1	0
4	Asperlactone	184.18	0.01	4	1	2
5	4-hydroxy-3-nitrosobenzamide	166.13	1.59	4	2	2
6	Salinosporamide A	313.77	1.46	5	2	4
7	Tropodithietic acid	212.25	2.02	3	1	1
8	L-Tenuazonic acid	197.23	1.26	4	2	3
9	Cremeomycin	194.14	2.02	5	1	2
10	Azetidomonamide B	192.22	0.51	4	1	3
11	Calidoustene A	376.44	2.06	6	2	5
12	Coronatine	319.39	2.69	5	2	6
13	Cyanogramide B	387.43	2.75	6	1	3
14	Nematophin	272.34	2.83	4	2	7
15	Rubiginone A2	336.34	2.73	5	1	1

MW—molecular weight; LogP—partition coefficient; HBD—hydrogen bond donors; HBA—hydrogen bond acceptors; RB—rotatable bonds. * Values within parentheses are the standard threshold considered for the fungicide screening.

Interaction analysis of the lead compound: An interaction analysis was performed for the 14 lead compounds including the reference to gain insights into molecular mechanisms underlying between target and ligand. The screened compounds exhibited various interactions including hydrogen bonding, hydrophobic interactions and salt bridge interactions. In this analysis, the reference compound (PubChem ID: 449402), when interacting with the target protein (PDB ID: 2STD), exhibited notable hydrogen bond interactions with TYR50 and hydrophobic interactions with key residues such as TYR30, PHE53, VAL75, LEU76, VAL108, HIS110, ALA127, ILE151, PHE158 and PHE162 (Figure 3a). Notably, hydrophobic interactions play a pivotal role in stabilizing protein-ligand complexes by contributing to binding affinity and specificity through the burial of nonpolar residues within the complex interior[19].

Table 3
Docking score and binding free energy contributions between 2STD and lead compounds

S.N.	PubChem / MIBiG ID	Compound	Tanimoto Coefficient	XP GScore (kcal/mol)	RF scoring (pKa)	KDEEP (kcal/mol)	Gnina (CNN affinity)
1	449402	Carpropamid (Reference)	1	-5.3	5.95	-6.55	4.35
2	89778	Shanorellin	0.32	-6.3	6.04	-6.91	6.04
3	114679	Mellein	0.39	-7.3	5.96	-6.85	5.96
4	156698	Asperlactone	0.35	-6.1	5.98	-6.59	5.98
5	443631	4-hydroxy-3-nitrosobenzamide	0.47	-6.1	5.98	-6.55	5.98
6	11695330	Salinosporamide A	0.33	-9.3	5.98	-10.43	5.98
7	44632924	Tropodithietic acid	0.38	-6.2	6.12	-6.57	6.12
8	54683011	L-Tenuazonic acid	0.38	-6.4	5.96	-8.24	5.96
9	54704722	Cremeomycin	0.35	-6.7	5.98	-7.57	5.98
10	NPA032934	Azetidomonamide B	0.38	-6.9	5.96	-6.92	5.96
11	NPA033273	Calidoustene A	0.32	-7.7	5.99	-7.18	5.99
12	NPA007302	Coronatine	0.33	-6.4	5.96	-6.76	5.96
13	NPA031550	Cyanogramide B	0.32	-7.2	5.96	-6.58	5.96
14	NPA007705	Nematophin	0.41	-8.6	5.96	-9.71	5.96
15	NPA033446	Rubiginone A2	0.34	-7.1	5.96	-6.59	5.96

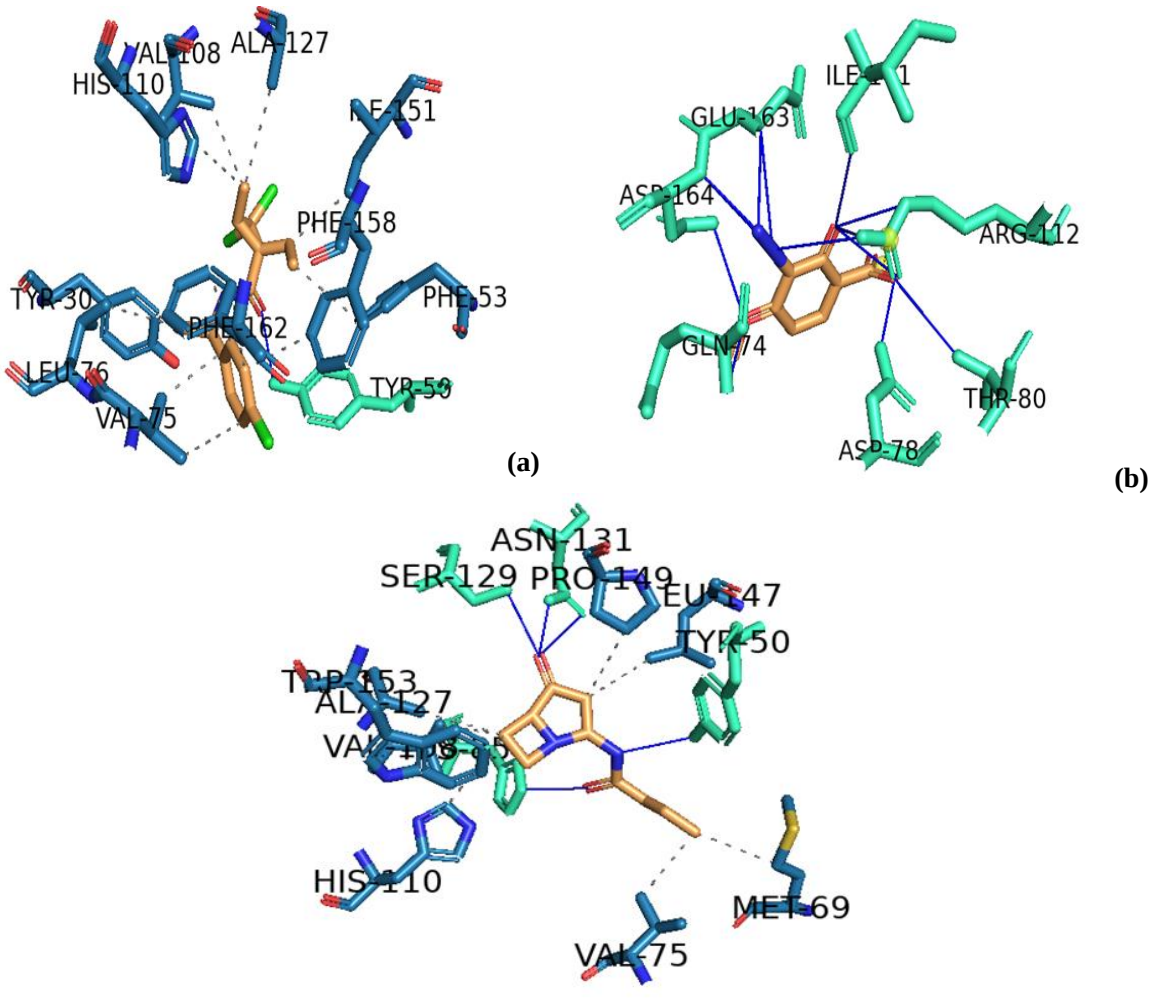

Figure 3: Interaction analysis of (a) Carpropamid (b) Cremeomycin (c) Azetidomonamide B with 2STD. Residues in green cyan form a hydrogen bond interaction with ligands. The yellow line indicates salt bridge interaction whereas grey lines are hydrophobic interactions

Table 4
Description of lead bioactive compounds from endophytes with their biosynthetic gene cluster type

S.N.	Compound	Identified isolates	Region No.	BGC type
1	Shanorellin	*S. albidoflavus*	1.16	NRPS, T1PKS, lanthipeptide-class-ii,transAT-PKS
		S. LBUM 1475	1.7	T1PKS
		S. lusitanus	1.13	T1PKS
		S. yangpuensis	1.11,	T1PKS
		S. pratensis	1.14	Terpene
		S. xanthii	1.23	NRPS
			1.1	2PKS, oligosaccharide
2	Mellein	*S. alboflavus*	1.2, 1.22	T1PKS
		S. genisteinicus	1.15,	T1PKS
			1.17	T1PKS, butyrolactone
		S. roseirectus	1.1,	T1PKS,
			1.2	T1PKS, arylpolyene
		S. CRSS-Y-16	1.14	NRPS, T1PKS
		S. CFMR 7 strain CF	1.7	NRPS, T1PKS
3	Asperlactone	*S. alboflavus*	1.2, 1.21, 1.22	T1PKS
		S. CRSS-Y-16	1.14	NRPS, T1PKS
		S.genisteinicus	1.15	T1PKS
		S. roseirectus	1.1,	T1PKS,
			1.2	T1PKS, arylpolyene
		S. A10	1.2	NRPS, T1PKS
		S. CFMR 7 strain CF	1.7,	NRPS, T1PKS
			1.8	T1PKS
4	4-hydroxy-3-nitrosobenzamide	*S. yangpuensis*	1.16	Melanin NRPS, T1PKS, hglE-KS, other
		S. pratensis	1.4,	Melanin
			1.14	Melanin
		S.broussonetiae	1.12	T3PKS
		S. alboflavus	1.4,	Melanin
			1.8,	Melanin
			1.10	Melanin
		S.genisteinicus	1.13	T2PKS
		S. roseirectus	1.19,	nucleoside, transAT-PKS
			1.24,	Melanin
			1.26	Melanin
		S. CRSS-Y-16	1.9	Melanin
		S. CFMR 7 strain CF	1.4,	NRP-metallophore, NRPS, melanin
			1.33,	Arylpolyene
			1.37	
5	Salinosporamide A	*S. pratensis*	1.12	NRPS, transAT-PKS
		S. HNM0140	1.1	Butyrolactone
6	Tropodithietic acid	*S. CRSS-Y-16*	1.21	terpene
7	L-Tenuazonic acid	*S.broussonetiae*	1.23	NI-siderophore,NRPS
		S. HSG2	1.18	NRP-metallophore,NRPS
8	Cremeomycin	*S. alboflavus*	1.26	Arylpolyene
		S. LRE541	1.23	NRPS, T3PKS
9	Azetidomonamide B	*S. pratensis*	1.12	NRPS,transAT-PKS
		S. CFMR 7 strain CF	1.25	NRP-metallophore, NRPS
10	Calidoustene A	*S. roseirectus*	1.23	T1PKS

11	Coronatine	*S. CFMR 7 strain CF* *S. HSG2*	1.12 1.18	NRPS NRP-metallophore,NRPS
12	Cyanogramide B	*S. roseirectus* *S. CFMR 7 strain CF* *S. LBUM 1475*	1.29, 1.38 1.9 1.15	T3PKS, NRPS, T1PKS thioamide-NRP Bottromycin
13	Nematophin	*S. albidoflavus* *S. alboflavus* *S. CFMR 7 strain CF*	1.9 1.5 1.24	NRPS, NRPS NRPS
14	Rubiginone A2	*S. S4.7*	1.4	T3PKS

Of the various lead compound, cremeomycin (PubChem ID: 54704722) demonstrated the maximum number of interactions. Notably, cremeomycin was engaged in hydrogen interactions with GLN74, ASP78, THR80, ARG112, ILE161 and GLU163, along with a salt bridge interaction with ARG112 (Figure 3b). Following closely behind, azetidomonamide B (NPAtlas ID: NPA032934) displayed hydrogen interactions with TYR50, HIS85, SER129 and ASN131, as well as hydrophobic interactions with MET69, VAL75, VAL108, HIS110, ALA127, LEU147, PRO149 and TRP153 (Figure 3c). Further, the fungicidal likeness properties were analysed in the scrutinized compounds.

Structural Motif Analysis for Determining Fungicidal Activity: Scaffold analysis simplifies the identification of patterns and clusters within a molecule by facilitating the examination of its structural framework. The interaction analysis revealed that cremeomycin identified from *Streptomyces alboflavus* and *Streptomyces sp. LRE541* had the highest number of interactions with the target protein. The carboxyl moiety of cremeomycin, which interacts with the SDH protein at THR80 and ASP78, is primarily responsible for its phytopathogenic effects. Carboxylic acid, an organic compound comprising the carboxyl group, exhibits diverse effects on phytopathogens including growth inhibition, cell membrane disruption and interference with metabolic processes. For instance, acetic acid, a form of carboxylic acid, demonstrates antifungal activity against *Fusarium oxysporum* by disrupting its cell membranes and inhibiting its growth[30].

Similarly, benzoic acid acts by interfering with the cellular processes of the fungus *Botrytis cinerea*[8] and citric acid disrupts the metabolic activity in *Alternaria alternata* to induce antifungal activity[30].

Furthermore, the enamide in metabolite Azetidomonamide B from *Streptomyces pratensis* and *Streptomyces sp. CFMR 7* displays promising effects on phytopathogens. Enamide interacts at HIS85, TYR50, VAL75 and MET69 residues of the protein. Similar to carboxylic acid, the enamide extracted from the rhizomes of *Zingiber zerumbet* exhibits antifungal activity against *Fusarium oxysporum*[11,32]. The enamide derivatives inhibit the growth of *Fusarium oxysporum* and reduce the symptoms that infected plants experience from *Fusarium* wilt[26]. Additionally, the enamide displays inhibitory effects against phytopathogens such as *Magnaporthe oryzae* and *Botrytis cinerea*[33].

Conclusion

The current study explores secondary metabolites derived from plant endophytic *Streptomyces* species against rice blast disease caused by *Magnaporthe oryzae*. The compound activity against SDH, a key player in melanin production was examined. Employing genome mining, molecular docking and machine learning-based scoring functions (ML-SF), the study identified 14 lead compounds with binding affinities superior to carpropamid, the reference compound. Notably, the carboxyl group in cremeomycin and the enamide moiety in azetidomonamide demonstrated promising interactions and fungicidal properties, as evidenced by extensive literature support. These findings suggest their potential as effective bio-fungicides.

Moving forward, further elucidating the mechanisms of action of the identified lead compounds and conducting field trials to assess their efficacy under real-world agricultural conditions, is crucial. Furthermore, the exploration of *Streptomyces*-based bio-fungicides and the formulation of sustainable disease management strategies will be pivotal for applications such as enhancing crop yield, promoting environmentally friendly agricultural practices and ensuring food security.

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