Front. Agr. Sci. Eng. 2018, **5**(2): 271–279
https://doi.org/10.15302/J-FASE-2018214
Available online at http://journal.hep.com.cn/fase

RESEARCH ARTICLE

Triggering the biocontrol of *Botrytis cinerea* by *Trichoderma harzianum* through inhibition of pathogenicity and virulence related proteins

Kandasamy SARAVANAKUMAR, Zhixiang LU, Hai XIA, Meng WANG, Jianan SUN, Shaoqing WANG, Qiang-qiang WANG, Yaqian LI, Jie CHEN (✉)

School of Agriculture and Biology/State Key Laboratory of Microbial Metabolism/Key Laboratory of Urban Agriculture (South) of the Ministry of Agriculture, Shanghai Jiao Tong University, Shanghai 200240, China

Abstract This study reports a strain of *Trichoderma harzianum* CCTCC-SBW0162 with potential to enhance biocontrol activity against gray mold pathogen, *Botrytis cinerea*, and with a pivotal role in tomato (*Solanum esculentum*) plant growth enhancement. A total of 254 *Trichoderma* isolates were screened by *in vitro* antagonistic assay. Of these, 10 were selected for greenhouse experiments based on their greater inhibition of *B. cinerea*. The *in vitro* antagonistic assay and greenhouse experiments indicated that *T. harzianum* CCTCC-SBW0162 gave the highest inhibition rate (90.6%) and disease reduction (80.7%). Also, to study the possible mechanism associated with antifungal activity of CCTCC-SBW0162 against *B. cinerea*, molecular docking was used to assess the interactions between CCTCC-SBW0162-derived metabolites, and pathogencity and virulence related proteins of *B. cinerea*. The molecular docking results indicated that the combination of harzianopyridone, harzianolide and anthraquinone C derived from CCTCC-SBW0162 could synergistically improve antifungal activity against *B. cinerea* through the inhibition/modification of pathogenicity and virulence related proteins. However, this computerized modeling work emphasized the need for further study in the laboratory to confirm the effect *T. harzianum*-derived metabolites against the proteins of *B. cinerea* and their interactions.

Keywords anthraquinone, *Botrytis cinerea*, harzianolide, harzianopyridone, molecular docking, *Trichoderma harzianum*

1 Introduction

Solanum esculentum, although technically a fruit, is one of the most economically important vegetable fruit cultivated worldwide. However, its productivity is substantially decreased by gray mold, foliar diseases, root rots and wilts by several phytopathogens[1–3]. The key phytopathogens belong to the genera *Botrytis, Fusarium, Macrophomina, Phytophthora, Rhizoctonia and Sclerotinia*[4]. Of these, *Botrytis* is particular highly hazardous to the tomato plants, causing gray mold disease[1]. Moreover, continued use of chemical fungicides is hazardous to the environment and can also lead to the resistance of the phytopathogens such as *Botrytis cinerea* and *B. fabae*[5]. Therefore, there is a clear need for an effective biocontrol agent to reduce the impact of *B. cinerea.* In this regard, several biocontrol microorganisms, such as *Bacillus*, *Pseudomonas* and *Trichoderma,* are available to reduce the impact of phytopathogens[6,7]. *Trichoderma* strains have potential to reduce gray mold rot[8–10] and can improve plant growth through production of plant hormones, vitamins, triggering plant immunity and nutrient uptake[11]. *Trichoderma harzianum* has received global attention as an effective biocontrol agent for several plant pathogens[12–15]. *T. harzianum* inhabits various ecological niches, such as soil, rhizosphere, lakes, forest sediments and coastal vegetation soil from agriculture or non-agriculture ecosystems[16]. This fungus also occurs in close association with plants and has been isolated from different substrata of the plants[17].

B. cinerea is ranked as the second most important phytopathogen in the world[1], and it causes severe plant diseases that result in significant loss of yield in economically important crop plants including beans, berries, grapes and tomato[18]. However, the underlying mechanism of disease incidence, virulence and pathogenicity of *B. cinerea* is not fully understood. The first and foremost

Received July 12, 2017; accepted November 24, 2017

Correspondence: jiechen59@sjtu.edu.cn

process of interaction is initiated through contact of the fungal cell wall with the host plant. Hence, the fungal cell wall is essential for the penetration, colonization and infection of the plant tissues[19] and the fungal cell wall proteins (glycoproteins) are involved in the host-pathogen interactions, virulence and pathogencity[20,21]. The genome of *B. cinerea* exhibits over 100 putative GPI (glycosylphosphatidylinositol) proteins and these cell wall glycoproteins are involved in pathogencity, virulence and host interaction of *B. cinerea*[21,22]. According to recent work, *bcpmr1* from the *B. cinerea* is essential for protein glycosylation, cell wall structure and virulence of *B. cinerea*. In addition, examination of *O*-linked glycosylation pathways demonstrated that the PMT genes were crucial for the fungal pathogencity[23].

Several workers have studied the pathogenesis and virulence of *B. cinerea*[24–30]. One of the findings indicated that *bcpmr1* encoded a P-type Ca^{2+}/Mn^{2+}-ATPase involved in protein glycosylation, cell wall structure and virulence of *B. cinerea*[30]. The identification of the function of the monocarboxylate is, however, reported from the mammalian metabolism rather than fungi. The presence of this monocarboxylate transporter BcMctA has been reported as being essential for *B. cinerea* pathogenicity[29]. Our study aimed to investigate the effects of *T. harzianum* and its metabolites on plant growth and enhanced biocontrol of *B. cinerea* through the modification/inhibition of the pathogenicity and virulence related proteins (*bcpmr1* encoding a P-type Ca^{2+}/Mn^{2+}-ATPase, BcMctA) of *B. cinerea.*

2 Materials and methods

2.1 Microorganisms

Trichoderma strains were isolated from a coastal wetland ecosystem[31], grown on modified potato dextrose agar (PDAm)[32] and preserved in 20% glycerol at −80°C. A culture of *B. cinerea* isolated from infected tomato leaves was obtained from the Center of *Trichoderma* Culture Collection of Shanghai Jiao Tong University (CCTCCSJ), China.

2.2 *In vitro* screening

In vitro antagonism of 254 *Trichoderma* isolates (T1-T254) against *B. cinerea* was tested by the dual culture method, as described by Dennis and Webster[33]. Percentage of mycelial growth was calculated by measuring the *B. cinerea* growth competing with *Trichoderma* isolates on PDAm in Petri dishes (9 cm).

2.3 *Trichoderma* enhanced plant growth

Seeds of tomato were surface-sterilized by the method of Huang et al.[34], and germinated on sterile wet-paper at room temperature for 4 d. The pre-germinated tomato seedlings were planted in a greenhouse in pots containing the natural agriculture soil, sterilized at 180°C for 6 h to remove other microbes, insects and weeds. The conidial suspension of *Trichoderma* and *B. cinerea* were prepared according to the method of Vinale et al.[35], and Nelson and Powelson[36]. The microbial inoculation of *Trichoderma*/pathogens on tomato seedlings was applied by spraying[37]. A 5 mL conidia suspension of *Trichoderma* (2.6×10^5 conidia per mL) and/or a 5 mL conidia suspension of *B. cinerea* (5×10^4 conidia per mL) were sprayed on tomato seedlings per treatment.

To analyze *Trichoderma* induced plant growth regulation, 12 treatments were applied: CK_1, uninoculated; CK_2, seedlings sprayed with *B. cinerea*; T1, seedlings sprayed with *Trichoderma atroviride* CCTCC-RW0008 and *B. cinerea*; T2, seedlings sprayed with *Trichoderma asperellum* CCTCC-RW0011 and *B. cinerea*; T3, seedlings sprayed with *T. harzianum* CCTCC-RW0006 and *B. cinerea*; T4, seedlings sprayed with *T. harzianum* CCTCC-SBW0162 and *B. cinerea*; T5, seedlings sprayed with *T. atroviride* CCTCC-RW0008 and *B. cinerea*; T6, seedlings sprayed with *T. atroviride* CCTCC-SBW0138 and *B. cinerea*; T7, seedlings sprayed with *T. aureoviride* CCTCC-SBW0122 and *B. cinerea*; T8, seedlings sprayed with *T. atroviride* CCTCC-SBW0074 and *B. cinerea*; T9, seedlings sprayed with *T. atroviride* CCTCC-SBW0068 and *B. cinerea*; and T10, seedlings sprayed with *T. atroviride* CCTCC-SBW0073 and *B. cinerea*. One month after microbial inoculation, the *Trichoderma* induced growth regulating indicators of tomato, including shoot length, root length, shoot biomass, root biomass and total biomass, were measured. *Botrytis* disease reduction was evaluated using the modified formula of Saravanakumar et al.[31]. Each treatment had three replicates in a randomized design.

2.4 Molecular interaction

Molecular interaction of *T. harzianum*-derived secondary metabolites and pathogenicity related proteins from *B. cinerea* were assessed with a computer-based molecular docking program. The presence of the known *T. harzianum* metabolites, including T22azaphilone, harzianopyridone, harzianolide, 1-hydroxy-3-methyl-anthraquinone and anthraquinone C[38], in CCTCC-SBW0162 was confirmed by preliminary biochemical experiments (data not shown) and subsequent detailed chemical characterization of compounds. Therefore, these compounds were used for the molecular interaction study. The ligand structures (metabolites) were obtained from PubChem (NCBI-PubChem Compund), and the ligand was prepared by using the ACD/ChemSketch.

Earlier reports showed that *bcpmr1* encodes a P-type Ca^{2+}/Mn^{2+}-ATPase, and *BcMctA* from *B. cinerea* is

involved in pathogenicity and virulence[29,30]. A BLAST analysis indicated that *BcMctA* is identical to MFS monocarboxylate transporter (CCD50452), and the *bcpmr1* is identical to Ca^{2+}/Mn^{2+}-transporting P-type ATPase PMR1 (NP_011348) and hypothetical protein SS1G_09885 (EDN94018). Therefore, in the present study, *bcpmr1* was considered identical to PMR1 (NP_011348) and SS1G_09885 (EDN94018), and *BcMctA* was identical to MFS (CCD50452)[29,30]. Hence, available protein sequences of *bcpmr1* and *BcMctA* for molecular interaction with *T. harzianum* metabolites were used, after retrieving the protein sequences from NCBI protein database and the protein structure were predicte-dusing SWISS-MODEL. Molecular docking was analyzed with ArgusLab 4.0.1, and interactions of the protein and ligand were visualized with BIOVIA Discovery Studio 2016 (Accelrys Software Inc., San Diego, CA, USA).

3 Results and discussion

3.1 *In vitro* antagonism

In vitro antagonistic experimental results indicated that mycelial growth of *B. cinerea* was significantly inhibited by *Trichoderma* isolates in dual culture. The percentage inhibition ranged from 1.56% to 90.6% with *T. harzianum* CCTCC-SBW0162 exhibiting the highest inhibition of *B. cinerea* (Table S1). Out of 254 *Trichoderma* isolates tested, the top 10 were selected for greenhouse experiments to assessing the *Trichoderma* induced enhanced growth in tomato (Fig. 1) based their high percentage of inhibition of *Trichoderma*. The selected isolates were *T. harzianum* RW0006 (81.3%), *T. harzianum* SBW0162 (90.6%), *T. atroviride* SBW0138 (76.6%), *T. aureoviride* SBW0122 (79.7%), *T. atroviride* SBW0074 (81.3%), *T. atroviride* SBW0073 (85.9%), *T. atroviride* SBW0068 (76.6%), *T. atroviride* SBW0008 (78.1%), *T. asperellum* RW0011 (84.4%) and *T. atroviride* RW0008 (75.0%).

3.2 Plant growth enhancement induced by *Trichoderma*

The effects of *Trichoderma* on reduction of *B. cinerea* and enhancement of tomato growth under greenhouse conditions are shown in Table 1 and Fig. S1. Average shoot length was significantly affected by the treatments and ranged from 18.6±2.5 to 41.4±2.6 cm. Shoot length was increased significantly by about 1.22 times in T4 compared

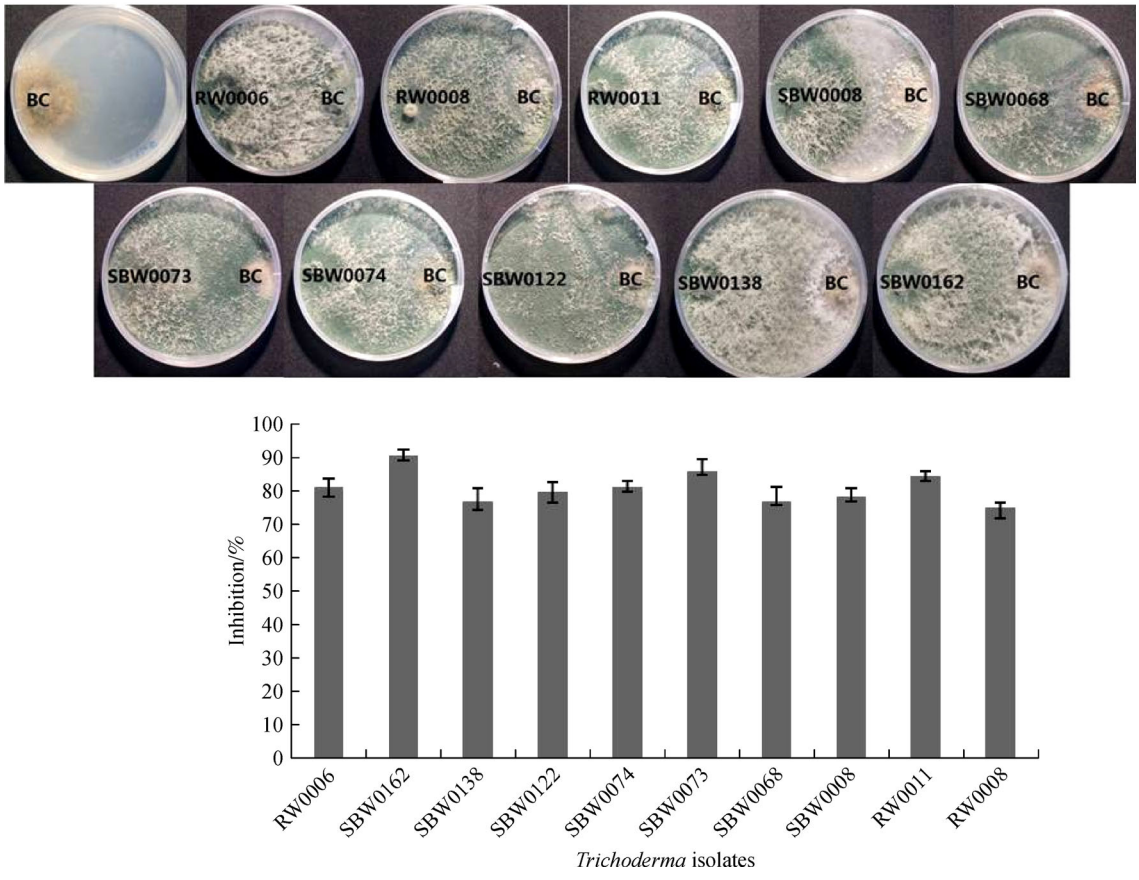

Fig. 1 *In vitro* antagonistic activity of *Trichoderma* against *B. cinerea* (BC) in dual culture: BC-CK, *T. harzianum* CCTCC-RW0006, *T. atroviride* CCTCC-RW0008, *T. asperellum* CCTCC-RW0011, *T. atroviride* CCTCC-SBW0008, *T. atroviride* CCTCC-SBW0068, *T. atroviride* CCTCC-SBW0073, *T. atroviride* CCTCC-SBW0074, *T. aureoviride* CCTCC-SBW0122, *T. atroviride* CCTCC-SBW0138, and *T. harzianum* CCTCC-SBW0162.

Table 1 Antagonistic effect of *Trichoderma* against *B. cinerea* on growth factors of tomato seedlings

Treatment	Average shoot length/cm	Average root length/cm	Average shoot biomass/g	Average root biomass/g	Total biomass/g	Disease reduction/%
CK_1	30.2±1.2 (0.62)	5.0±1.2 (0.56)	1.0±0.02 (4.0)	0.4±0.08 (1.0)	1.4±0.3 (2.50)	56.7
CK_2	18.6±2.5	3.2±0.8	0.2±0.01	0.2±0.09	0.4±0.1	31.7
T1	32.0±2.1 (0.72)	3.6±0.1 (0.13)	0.9±0.03 (3.5)	0.3±0.05 (0.5)	1.1±0.6 (0.75)	50.0
T2	29.7±1.5 (0.59)	4.2±0.6 (0.31)	0.2±0.02 (0.0)	0.2±0.06 (0.0)	0.4±0.8 (0.00)	40.0
T3	37.8±3.2 (1.03)	22.1±0.2 (5.90)	2.6±0.01 (12.0)	0.5±0.06 (1.5)	3.1±0.4 (6.75)	40.0
T4	41.4±2.6 (1.22)	11.5±0.4 (2.59)	3.8±0.03 (18.0)	1.6±0.04 (7.0)	5.4±0.5 (12.50)	80.7
T5	24.4±1.2 (0.31)	8.6±0.6 (1.68)	1.8±0.06 (8.0)	0.6±0.06 (2.0)	2.4±0.6 (5.00)	33.3
T6	32.4±0.6 (0.74)	6.5±1.2 (1.03)	3.4±0.06 (16.0)	0.2±0.04 (0.0)	3.6±0.2 (8.00)	56.7
T7	29.5±1.8 (0.58)	12.2±1.4 (3.00)	2.7±0.04 (2.5)	0.3±0.06 (0.5)	2.9±0.1 (1.50)	46.7
T8	20.0±1.6 (0.07)	3.5±0.6 (0.09)	1.2±0.06 (5.0)	0.5±0.01 (1.5)	1.7±0.2 (6.25)	33.3
T9	37.7±2.4 (1.02)	4.5±1.8 (0.40)	2.4±0.04 (11.0)	0.4±0.02 (1.0)	2.8±0.6 (6.00)	60.0
T10	32.6±1.6 (0.75)	7.0±1.6 (1.18)	1.9±0.09 (8.5)	1.1±0.03 (4.5)	3.0±0.1 (6.50)	56.7

Note: The values shown are means±SE (df = 60 seedlings per treatment) and one way ANOVA followed by multiple comparison using Duncan's test. The values in parentheses are the relative increase in treatment compared to negative control inoculated with *B. cinerea* and without *Trichoderma* treatment. CK_1, uninoculated sterile soil; CK_2, soil inoculated with *B. cinerea*; T1–T10, inoculated with different *Trichoderma* strains and *B. cinerea* as detailed in the materials and methods.

to the CK_2. Average root length varied with the treatments and it increased significantly 5.9 times in T3 when compared to CK_2. Average shoot biomass (18 times; $P < 0.05$), average root biomass (7 times; $P < 0.05$) and total biomass (12.5 times; $P < 0.05$) increased significantly in T4 compared to CK_1. Disease reduction was significant between the treatments ($P < 0.05$) with greatest reduction (80.7%) in T4 compared to (31.7%) in CK_2. Thus, treatment T4 showed that *T. harzianum* CCTCC-SBW0162 significantly reduce gray mold and improved tomato growth, which is consistent with other reports that *Trichoderma* spp. promote plant growth[39].

3.3 Generation of protein and metabolite structures

The predicted protein structures of *bcpmr1* [PMR1 (NP_011348) and SS1G_09885 (EDN94018)] and *BcMctA* [MFS (CCD50452)] are shown in Fig. 2. The structure of *T. harzianum* metabolites (ligand) such as T22azaphilone, harzianopyridone, harzianolide, 1-hydroxy-3-methyl-anthraquinone, and 1, 8-dihydroxy-3-methyl-anthraquinone are shown in Fig. 3.

3.4 Molecular interaction studies

A total of three target proteins were tested for interactions with *T. harzianum*-derived compounds and the results indicated that they can have a significant inhibitory effect against pathogenicity and virulence related proteins of *B. cinerea* (Table 2). Secondary metabolites produced from *T. harzianum* are known to inhibit the pathogens such as *Gaeumannomyces graminis, Pythium ultimum* and *Rhizoctonia solani in vitro*[38]. Similarly the present work indicated that the *T. harzianum*-derived metabolites can

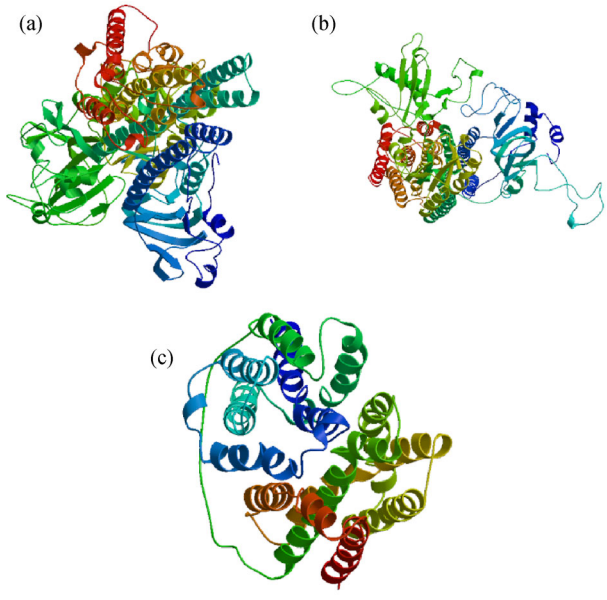

Fig. 2 3D structure of target protein of *B. cinerea*. *Bcpmr1* is identical to PMR1 (NP_011348) (a) and SS1G_09885 (EDN94018) (b), and *BcMctA* is identical to MFS (c).

inhibit the growth of *B. cinerea* through the inhibition/modification of pathogenicity and virulence proteins. The antifungal effect of *T. harzianum*-derived metabolites significantly varied between different types of phytopathogens[35]. Similarly, the present study indicated that among the five tested compounds anthraquinone C can provide the greatest inhibition of *bcpmr1* (−43.91 and −49.47 kJ·mol^{-1} for PMR1 and SS1G, respectively) and *BcMctA* (−57.71 kJ·mol^{-1}) than the other compounds tested for docking energy.

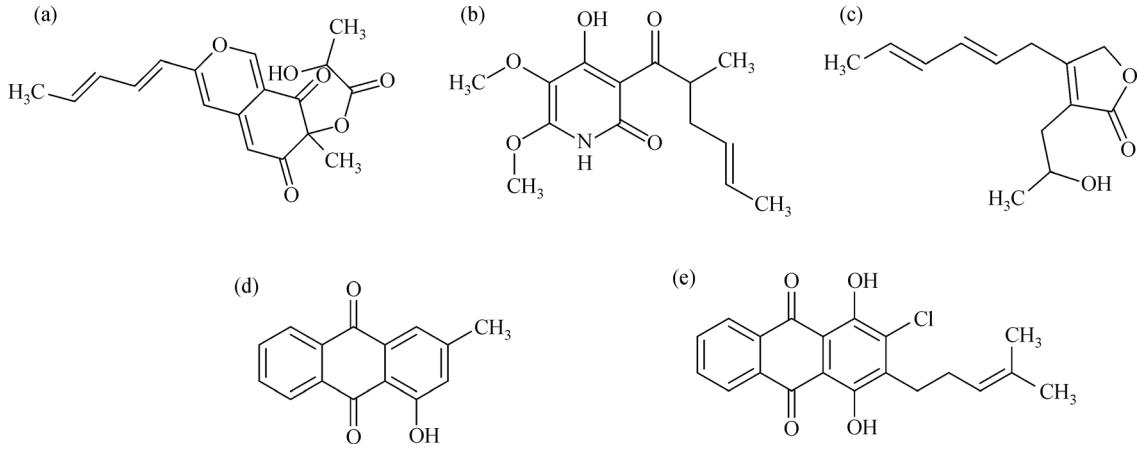

Fig. 3 Structure of metabolites (ligand) of *T. harzianum*. (a) T22azaphilone; (b) harzianopyridone; (c) harzianolide; (d) 1-hydroxy-3-methyl-anthraquinone; (e) 1,8-dihydroxy-3-methyl-anthraquinone.

Table 2 Analysis of interactions between *Trichoderma*-derived compounds and pathogenicity related protein *bcpmr1* of *B. cinerea*

S. No.	PubChem CID	*T. harzianum* derived Compound Name	Mol. Formula	Mol. Wt/(g·mol^{-1})	Docking Score/(kJ·mol^{-1})		
					Bcpmr		*BcMctA*
					PMR1	SS1G	MFS
1	76326344	T22azaphilone	$C_{18}H_{18}O_6$	330.336	−38.56	−42.57	−42.87
2	54697782	Harzianopyridone	$C_{14}H_{19}NO_5$	281.308	−40.36	−35.34	−38.39
3	15719532	Harzianolide	$C_{13}H_{18}O_3$	222.284	−40.27	−43.87	−45.17
4	164982	1-hydroxy-3-methyl-anthraquinone	$C_{15}H_{10}O_3$	238.242	−38.22	−41.65	−48.13
5	641293	anthraquinone C	$C_{20}H_{17}ClO_4$	356.802	−43.91	−49.47	−57.71

3.5 Molecular interaction of *Bcpmr* (PMR1 and SS1G_09885) with *T. harzianum*-derived metabolites

The examination of five candidate compounds of *T. harzianum* for molecular interaction indicated that they all had significant ability to inhibit and/or modify the pathogenicity and virulence related proteins *Bcpmr* (PMR1) of *B. cinerea* with strong docking scores from −38.22 to −43.91 kJ·mol^{-1} (Table 2). Among the compounds, anthraquinone C showed the highest docking score of −43.91 kJ·mol^{-1} with a strong interaction with hydrogen residues such as Arg182 and Asp703. Other residues were observed in the binding pockets such as Glu75, Gly241, Gly263, Ile76, Leu148 Phe262, Phe266, Thr702, Val704, Val682 and (Fig. 4). This interaction strongly indicated the potential of anthraquinone C to modify targeted protein structures and such modification could change protein function.

The results indicated that all the tested compounds showed significant potential interactions with the SS1G_09885; and among the tested compounds anthraquinone C had the highest docking score of −49.47 kJ·mol^{-1} with strong interactions with hydrophobic residues, such as Met447, Gln444 and Met520, and other residues, such as Glu524, Ile826, Ile830, Leu834, Leu495, Leu503, Leu443, Pro521, Thr441, Val523, Phe827, and Val499 (Fig. 5). This demonstrates that the fungal metabolites could have a significant ability to interact with and interfere with the functioning of Bcpmr-encoded protein.

3.6 Molecular interaction of *BcMctA* (MFS) with *Trichoderma harzianum*-derived compounds

BcMctA is significantly involved in the pathogenicity and virulence of *B. cinerea* and it was found that among the tested compounds, anthraquinone C could interact significantly with the *BcMctA* protein with a docking score of −57.71 kJ·mol^{-1} and strong interaction with protein residues such as Leu111, Phe114, Ser110, Leu404, Phe401 and Phe117 (Fig. 6). All the docking scores of *T. harzianum*-derived compounds with pathogenicity and virulence related proteins in *B. cinerea* indicated the potential of *T. harzianum* to inhibit *B. cinerea* by targeting this protein. Several researchers have reported a potential reduction of protein function as evidenced by negative docking scores from the computational method using ArgusLab[35,40–43] and the present results suggest that

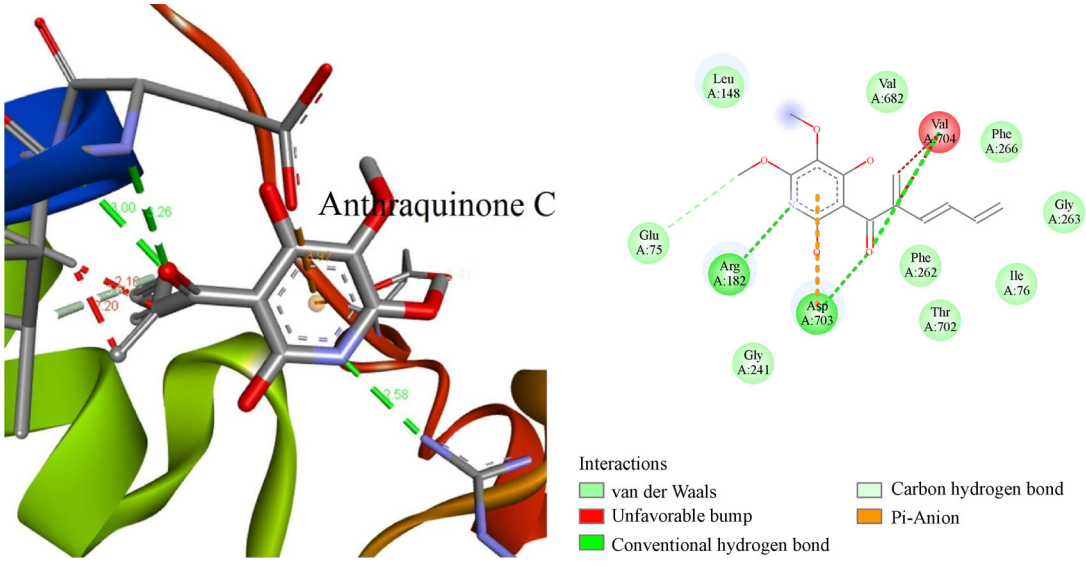

Fig. 4 Docking complex showing the interaction between *Trichoderma* metabolites anthraquinone C with *Bcpmr* (PMR1) of *B. cinerea*

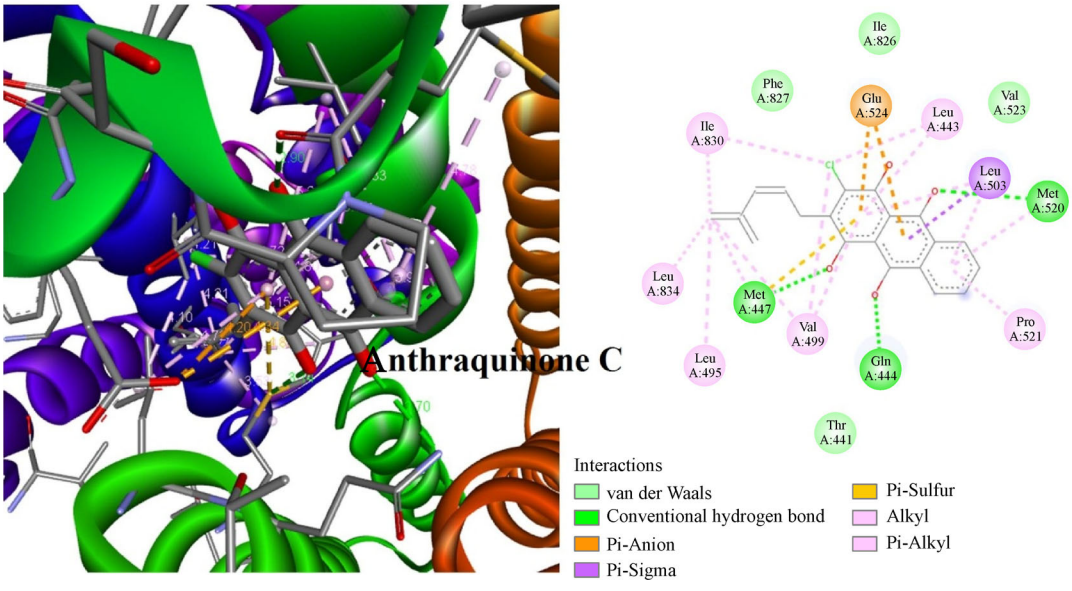

Fig. 5 Docking complex showing the interaction between *Trichoderma* metabolite, anthraquinone C, with *Bcpmr* (SS1G_09885) of *B. cinerea*

anthraquinone C could interact significantly with the *BcMctA* protein and inhibit its functioning.

Harzianopyridone, harzianolide and anthraquinone C are known to reduce the gray mold disease in tomato caused by *B. cinerea*[29,35]. The negative docking scores for these three compounds (Table 2) indicated that they could work synergistically against *B. cinerea* and inhibit its growth through targeting the pathogenicity and virulence related proteins. These molecular docking results indicate that some secondary metabolites from *Trichoderma* spp., could potentially interact strongly with phytopathogens. However further detailed laboratory and greenhouse studies are required to confirm this.

4 Conclusions

This work reports a potent *Trichoderma* strain with potentially useful biocontrol activity against the gray mold pathogen, *B. cinerea,* investigated by *in vitro* and

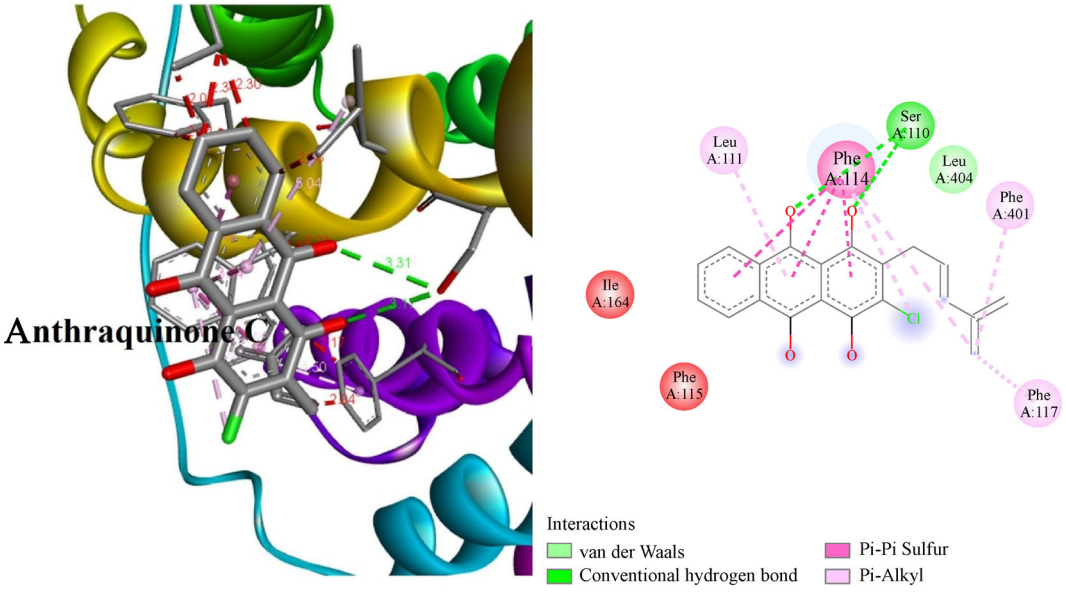

Fig. 6 Docking complex showing the interaction between *Trichoderma* metabolite, anthraquinone C, with *BcMctA* (MFS) of *B. cinerea*

greenhouse experiments. Significant potential molecular interactions between *T. harzianum*-derived metabolites and pathogenicity, virulence related proteins of *B. cinerea* indicated that the inhibition of the *B. cinerea* may not triggered by single metabolite, but is likely to be a synergistic effect of multiple metabolites from *T. harzianum*. Notably, the negative docking score for anthraquinone C indicated it could have the greatest ability to inhibit *B. cinerea*, and the combination of harzianopyridone, harzianolide and anthraquinone C may also increase the potential biocontrol activity of *T. harzianum* against *B. cinerea*. Taken together, these findings provide important new information about the molecular interactions of metabolites and pathogenicity-related virulence proteins. This contrasts with established methods which are generally based on trial and error testing, with a low probability of success in the laboratory experiments[44]. Although this study needs to be confirmed by further study of the interaction of *T. harzianum* metabolites with *B. cinerea* proteins in a microbiology laboratory, this approach should lead to a greater likelihood of success.

Supplementary materials The online version of this article at https://doi.org/10.15302/J-FASE-2018214 contains supplementary materials (Table S1; Fig. S1).

Acknowledgements This study was supported by the National Key Research and Development Program of China (2017YFD0200400, 2017YFD0201108, SQ2017ZY06102), the National Natural Science Foundation of China (20090073110048), the National Modern Agriculture Industry Technique Systems (CARS-02), Special Project of Basic Work Project for Science and Technology (2014FY120900).

Compliance with ethics guidelines Kandasamy Saravanakumar, Zhixiang Lu, Hai Xia, Meng Wang, Jianan Sun, Shaoqing Wang, Qiang-qiang Wang, Yaqian Li, and Jie Chen declare that they have no conflicts of interest or financial conflicts to disclose.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Dean R, Van Kan J A, Pretorius Z A, Hammond-Kosack K E, Di Pietro A, Spanu P D, Rudd J J, Dickman M, Kahmann R, Ellis J, Foster G D. The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, 2012, **13**(4): 414–430
2. Montealegre J R, Herrera R, Velasquez J C, Silva P, Besoain X, Perez L M. Biocontrol of root and crown rot in tomatoes under greenhouse conditions using *Trichoderma harzianum* and *Paenibacillus lentimorbus*. Additional effect of solarization. *Electronic Biotechnology*, 2005, **8**(3): 249–257
3. Srinon W, Chuncheen K, Jirattiwarutkul K, Soytong K, Kanokmedhakul S. Efficacies of antagonistic fungi against *Fusarium wilt* disease of cucumber and tomato and the assay of its enzyme activity. *Agricultural Technology*, 2006, **2**(2): 191–201
4. Talla S G, Raju A S R, Karri S, Kumar Y S. Production and antagonistic effect of *Trichoderma* spp. on pathogenic microorganisms (*Botrytis cinerea, Fusarium oxysporium Macrohomina phasealina* and *Rhizoctonia solani*). *African Journal of Biotechnology*, 2015, **14**(8): 668–675
5. Parry D W. Diseases of potato. In: Plant pathology in agriculture. Cambridge, UK: *Cambridge University Press*, 1990
6. Weller D M, Raaijmakers J M, Gardener B B M, Thomashow L S. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology*, 2002, **40**(1): 309–348
7. Huang H C, Erickson R S, Chang C, Moyer J R, Larney F J, Huang J W. Control of white mold of bean caused by *Sclerotinia*

sclerotiorum using organic soil amendments and biocontrol agents. *Plant Pathology Bulletin*, 2005, **14**(3): 183–190

8. Harman G E, Latorre B, Agosin E, Martin R S, Riegel D G, Nielsen P A, Tronsmo A, Pearson R C. Biological and integrated control of *Botrytis* bunch rot of grape using *Trichoderma* spp. *Biological Control*, 1996, **7**(3): 259–266
9. Zimand G, Elad Y, Chet I. Effect of *Trichoderma harzianum* on *Botrytis cinerea* pathogenicity. *Phytopathology*, 1996, **86**(11): 1255–1260
10. Fravel D R, Rhodes D J, Larkin R P. Biological control of diseases: production and commercialization of biocontrol products. In: Clercq P D. Integrated Pest and Disease Management in Greenhouse Crops. Wageningen: *Springer Netherlands*, 1999, 365–376
11. Joshi B B, Bhatt R P, Bahukhandi D. Antagonistic and plant growth activity of *Trichoderma* isolates of Western Himalayas. *Journal of Environmental Biology*, 2010, **31**(6): 921–928
12. Benítez T, Rincón A M, Limón M C, Codón A C. Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology*, 2004, **7**(4): 249–260
13. Soytong K, Srinon W, Rattanacherdchai K, Kanokmedhakul S, Kanokmedhakul K. Application of antagonistic fungi to control anthracnose disease of grape. *Journal of Agricultural Biotechnology*, 2005, **1**: 33–41
14. Morsy E M. Role of growth promoting substances producing microorganisms on tomato plant and control of some root rot fungi. Dissertation for the Doctoral Degree. Cairo: *Ain shams University*, 2005
15. Zaghloul R A, Hanafy Ehsan A, Neweigy N A, Khalifa Neamat A. Application of biofertilization and biological control for tomato production. In: 12th Conference of Microbiology 2005, Cairo. Egypt: *Factory of Agriculture, Benha University*, 2007, 198–212
16. Saravanakumar K, Yu C, Dou K, Wang M, Li Y, Chen J. Biodiversity of *Trichoderma* community in the tidal flats and wetland of southeastern China. *PLoS One*, 2016, **11**(12): e0168020
17. Hermosa R, Botella L, Keck E, Jiménez J A, Montero-Barrientos M, Arbona V, Gómez-Cadenas A, Monte E, Nicolás C. The overexpression in *Arabidopsis thaliana* of a *Trichoderma harzianum* gene that modulates glucosidase activity, and enhances tolerance to salt and osmotic stresses. *Journal of Plant Physiology*, 2011, **168** (11): 1295–1302
18. Williamson B, Tudzynski B, Tudzynski P, van Kan J A. Botrytis cinerea: the cause of grey mould disease. *Molecular Plant Pathology*, 2007, **8**(5): 561–580
19. Cantu D, Vicente A R, Greve L C, Dewey F M, Bennett A B, Labavitch J M, Powell A L T. The intersection between cell wall disassembly, ripening, and fruit susceptibility to *Botrytis cinerea. Proceedings of the National Academy of Sciences of the United States of America*, 2008, **105**(3): 859–864
20. González C, Brito N, Sharon A. Infection process. Fungal virulence factors. In: S. Fillinger, Y. Elad, eds. *Botrytis*—the Fungus, the Pathogen and Its Management in Agricultural Systems. Heidelberg: *Springer International Publishing*, 2015, 229–246
21. González M, Brito N, Frías M, González C. Botrytis cinerea protein O-mannosyltransferases play critical roles in morphogenesis, growth, and virulence. *PLoS One*, 2013, **8**(6): e65924
22. De Groot P W. A genomic inventory of cell wall biosynthesis in the ubiquitous plant pathogen *Botrytis cinerea*. In: Mora-Montes, eds. The Fungal Cell Wall. Hauppauge: *Nova Biomedical*, 2013
23. González M, Brito N, González C. High abundance of Serine/Threonine-rich regions predicted to be hyper-*O*-glycosylated in the secretory proteins coded by eight fungal genomes. *BMC Microbiology*, 2012, **12**(1): 213
24. Michielse C B, Becker M, Heller J, Moraga J, Collado I G, Tudzynski P. The *Botrytis cinerea* Reg1 protein, a putative transcriptional regulator, is required for pathogenicity, conidiogenesis, and the production of secondary metabolites. *Molecular Plant-Microbe Interactions*, 2011, **24**(9): 1074–1085
25. Aguayo C, Riquelme J, Valenzuela P D T, Hahn M, Moreno E S. Bchex virulence gene of *Botrytis cinerea*: characterization and functional analysis. *Journal of General Plant Pathology*, 2011, **77** (4): 230–238
26. Giesbert S, Schumacher J, Kupas V, Espino J, Segmüller N, Haeuser-Hahn I, Schreier P H, Tudzynski P. Identification of pathogenesis-associated genes by T-DNA-mediated insertional mutagenesis in *Botrytis cinerea*: a type 2A phosphoprotein phosphatase and an SPT3 transcription factor have significant impact on virulence. *Molecular Plant-Microbe Interactions*, 2012, **25**(4): 481–495
27. Harren K, Schumacher J, Tudzynski B. The Ca^{2+}/calcineurin-dependent signaling pathway in the gray mold *Botrytis cinerea*: the role of calcipressin in modulating calcineurin activity. *PLoS One*, 2012, **7**(7): e41761
28. Yang Q, Chen Y, Ma Z. Involvement of BcVeA and BcVelB in regulating conidiation, pigmentation and virulence in *Botrytis cinerea. Fungal Genetics and Biology*, 2013, **50**(1): 63–71
29. Cui Z, Gao N, Wang Q, Ren Y, Wang K, Zhu T. BcMctA, a putative monocarboxylate transporter, is required for pathogenicity in *Botrytis cinerea. Current Genetics*, 2015, **61**(4): 545–553
30. Plaza V, Lagües Y, Carvajal M, Pérez-García L A, Mora-Montes H M, Canessa P, Larrondo L F, Castillo L. bcpmr1 encodes a P-type Ca^{2+}/Mn^{2+}-ATPase mediating cell-wall integrity and virulence in the phytopathogen *Botrytis cinerea. Fungal Genetics and Biology*, 2015, **76**: 36–46
31. Saravanakumar K, Yu C, Dou K, Wang M, Li Y, Chen J. Synergistic effect of *Trichoderma*-derived antifungal metabolites and cell wall degrading enzymes on enhanced biocontrol of *Fusarium oxysporum* f. sp. *Cucumerinum. Biological Control*, 2016, **94**: 37–46
32. Vargas Gil S, Pastor S, March G J. Quantitative isolation of biocontrol agents *Trichoderma* spp., *Gliocladium* spp. and *actinomycetes* from soil with culture media. *Microbiological Research*, 2009, **164**(2): 196–205
33. Dennis C, Webster J. Antagonistic properties of species groups of *Trichoderma* II. Production of non-volatile antibiotics. *Transactions of the British Mycological Society*, 1971, **57**(1): 41–48
34. Huang X, Zhang N, Yong X, Yang X, Shen Q. Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with *Bacillus pumilus* SQR-N43. *Microbiological Research*, 2012, **167**(3): 135–143
35. Vinale F, Sivasithamparam K, Ghisalberti E L, Marra R, Barbetti M J, Li H, Woo S L, Lorito M. A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiological and*

Molecular Plant Pathology, 2008, **72**(1): 80–86

36. Nelson M E, Powelson M L. Biological control of gray mold of snap beans by *Trichoderma hamatum. Plant Disease*, 1988, **72**(8): 727–729
37. Elad Y. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection*, 2000, **19**(8): 709–714
38. Vinale F, Marra R, Scala F, Ghisalberti E L, Lorito M, Sivasithamparam K. Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. *Letters in Applied Microbiology*, 2006, **43**(2): 143–148
39. Inbar J, Abramsky M, Cohen D, Chet I. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. *European Journal of Plant Pathology*, 1994, **100**(5): 337–346
40. Saravanakumar K, Li Y, Yu C, Wang Q Q, Wang M, Sun J, Gao J X, Chen J. Effect of *Trichoderma harzianum* on maize rhizosphere microbiome and biocontrol of *Fusarium* Stalk rot. *Scientific Reports*, 2017, **7**(1): 1771
41. Ferreira L G, Dos Santos R N, Oliva G, Andricopulo A D. Molecular docking and structure-based drug design strategies. *Molecules*, 2015, **20**(7): 13384–13421
42. Rudnitskaya A, Török B, Török M. Molecular docking of enzyme inhibitors: a computational tool for structure-based drug design. *Biochemistry and Molecular Biology Education*, 2010, **38**(4): 261–265
43. Doman T N, McGovern S L, Witherbee B J, Kasten T P, Kurumbail R, Stallings W C, Connolly D T, Shoichet B K. Molecular docking and high-throughput screening for novel inhibitors of protein tyrosine phosphatase-1B. *Journal of Medicinal Chemistry*, 2002, **45**(11): 2213–2221
44. Chaudhary N, Sandhu P, Ahmed M, Akhter Y. Structural basis of transport function in major facilitator superfamily protein from *Trichoderma harzianum. International Journal of Biological Macromolecules*, 2017, **95**: 1091–1100