Antagonistic and Biocontrol Potential of *Trichoderma asperellum* ZJSX5003 Against the Maize Stalk Rot Pathogen *Fusarium graminearum*

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Received: 8 January 2016 / Accepted: 1 April 2016 / Published online: 18 April 2016
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Abstract The efficacy of seven strains of *Trichoderma asperellum* collected from the fields in Southern China was assessed against *Fusarium graminearum* (FG) the causal agent of corn stalk rot of maize were in vitro for their antagonistic properties followed by statistical model of principal component analysis to identify the beneficial antagonist *T. asperellum* strain. The key factors of antagonist activity were attributed to a total of 13 factors including cell wall degrading enzymes (chitinase, protease and β -glucanases), secondary metabolites and peptaibols and these were analyzed from eight strains of *Trichoderma*. A linear regression model demonstrated that interaction of enzymes and secondary metabolites of *T. asperellum* strain ZJSX5003 enhanced the antagonist activity against FG. Further, this strain displayed a disease reduction of 71 % in maize plants inoculated with FG compared to negative control. Pointing out that the *T. asperellum* strain ZJSX5003 is a potential source for the development of a biocontrol agent against corn stalk rot.

Keywords Cell wall-degrading enzymes · Corn stalk rot · *Fusarium graminearum* · Principal component analysis · *Trichoderma asperellum*

Introduction

Maize is one of the important food crops worldwide. However, growing this crop finds difficulty due to its susceptibility to plant diseases caused by phytopathogens. Corn stalk rot (CSR) caused by the soil borne fungus *Fusarium graminearum* Schwabe (FG) (Teleomorph, *Giberella zeae* (Schwein) Petch is one of most serious issues worldwide, causing severe losses of maize production. The occurrence of CSR has been reported in 23 countries, including the United States, Canada, India, and France [1]. Use of the most chemical fungicides as seed coatings could not effectively control CSR due to the natural degradation of chemicals and non beneficial *Fusarium* sp., which can infect the maize at any growing stage and season.

Use of fungicides potentially increases the consequence of toxicity of environmental concern and little effect on biological control. Therefore, the use of biocontrol agents (BCAs) capable of colonising in the rhizosphere could constitute a potential disease management strategy for maize cultivation [2]. Therefore, farmers are interested to use the highly virulent microbial fungicides as BCAs against CSR. *Trichoderma* spp., are a well known BCAs group that control the pathogens through competition, antibiosis, mycoparasitism, besides inducing the plant defence responses and triggering plant growth by producing growth hormones, antibiosis compounds and cell wall degrading enzymes which act synergistically against the fungal pathogens [2–5]. In addition, some *Trichoderma* (Teleomorph, *Hypocrea*) strains can colonize corn roots during the juvenile phase and

Electronic supplementary material The online version of this article (doi:10.1007/s12088-016-0581-9) contains supplementary material, which is available to authorized users.

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induce broad spectrum systemic resistance responses (ISR) in leaves during later periods of plant growth [6].

Mycoparasitism involves direct antagonism against soil-borne pathogens through enzymatic lysis by secretion of cell wall degrading enzymes (CWDEs) such as chitinases, glucanases, and proteases [7]. Reportedly *Trichoderma* can produce the diverse range of secondary metabolites, CWDEs and peptaibols which enhance antagonism against fungal pathogens [8]. Production of peptaibols, a class of non-ribosomally synthesised linear peptides with antimicrobial properties by *Trichoderma virens* (Miller, Giddens and Foster) Arx and cloning of the gene responsible for its production has been established [9]. Numbers of studies dedicated to biological control of plant pathogens by *Trichoderma*, [10] have identified the production of secondary metabolites such as T22-azaphilone, harzianolide and T39 butenolide inhibitory to the *R. solani* (Teleomorph: *Thanatephorus* spp.), *P. ultimum* and *G. graminis* var. *tritici*. *Trichoderma harzianum* Rafai T22 colonising roots of maize seedlings. These metabolites may induce the expression of resistance-response genes, such as chitinase, β -1,3-glucanase and protease and are repressive to the effect on *Pythium ultimum* infecting the maize [2].

The search of novel *Trichoderma* strains with high biocontrol potential for plant disease management is required for the ubiquitous occurrence of *Trichoderma*. It is highly necessary to screen the candidate *Trichoderma* strains for potential control of CSR in corn seedlings. Therefore, the present study aimed at screening the potent *Trichoderma* strain against FG by in vitro, in planta greenhouse assays followed by confirming the antagonistic virulence through the study of enzymes and secondary metabolites from the potent *Trichoderma* strain.

Materials and Methods

Fungi Strains, Medium, and Culture Conditions

A total of seven different *Trichoderma asperellum* (Samuels, Liechfeldt et Nirenberg) strains collected from the fields in Southern China were selected for the present study based on antagonistic study [11] (Supplementary Table 1). *Trichoderma harzianum* SH2303 (CGMCC No. 4963) which has been characterized as potent BCAs [12] was used as a positive control in antagonist and greenhouse experiments for comparison. A virulent plant pathogen *F. graminearum* Schwabe (CGMCC No. 304598) originated from diseased maize was used for antagonistic experiments. The *Trichoderma* strains were maintained in potato dextrose agar (PDA) and stored as glycerol stocks at -80°C for genetic stability and viability. For the experimental purpose, agar plugs of *Trichoderma* strains were

inoculated in the PDA agar plates, and incubated at 28°C with 60 % humidity and continuous light for 5 days.

In Vitro Antagonist Assay

The in vitro antagonist activity against *F. graminearum* (FG) was determined using the dual culture technique [13]. Mycelial discs of 5 mm diameter removed from the growing edge of one week old *Trichoderma* and one week old FG culture were placed on the opposite sides of Petri dishes (90×15 mm) containing potato-dextrose agar at equal distance. A complete randomized experimental design was used with four Petri dishes for each antagonist. In control plates (without *Trichoderma*), a sterile agar disc was placed in place of the pathogen. The plates were incubated at $28 \pm 2^{\circ}\text{C}$ for 5 days in the dark and at the end of the incubation period, the diameter of mycelia growth was measured and used to determine percentage of inhibition by using the formula

$$I = (C - T)/C \times 100.$$

where *I* percent inhibition; *C* radial growth of pathogen (mm) alone (control); *T* radial growth of pathogen (mm) in the presence of *Trichoderma* strains.

Determination of Cell Wall-Degrading Enzyme Activity

The cell wall-degrading enzymes (CWDEs) such as chitinase, β -1,3-glucanase, and protease were determined from culture filtrate of different *T. asperellum* strains. In brief, the *Trichoderma* strain was cultured in liquid production medium consisted of peptone (0.1 %), Urea (0.03 %), KH_2PO_4 (0.2 %), $(\text{NH}_4)_2\text{SO}_4$ (0.14 %), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.03 %), $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ (0.03), and 1 ml of trace element solution (Fe^{2+} , Mn^{2+} , Zn^{2+} , and Co^{2+}), pH (5.5), for 72 h at 180 RPM. After the incubation, the culture filtrates were centrifuged at 3000 RPM for 10 min and the supernatants were collected and used for enzyme assay. 0.05 % of substrate such 1.0 % of colloidal chitin [15] or cellulase carboxymethyl cellulose or gelatin, or pachyman to detect the enzymes chitinase [14] or cellulase or protease [17] or β (1–3) glucanase [16] respectively were dissolved in 50 mM of sodium phosphate buffer (pH 7.0). One unit of the enzyme activity is defined as the required amount of enzymes to catalyse μg of reducing sugar per ml per minute under the respective reaction condition. All the measurements were performed in triplicates.

Extraction and Purification of Peptaibols

For peptaibol profiling, each strain was grown in a 250 ml flask dispensed with 50 ml of a mineral medium containing

(g l⁻¹) glucose (5.0), KH₂PO₄ (0.8), KNO₃ (0.7), Ca(H₂PO₄) (20.2), MgSO₄·7H₂O (0.5), MnSO₄·5H₂O (0.01), CuSO₄·5H₂O (0.005), FeSO₄·7H₂O (0.001) at 28 °C for 20 days. The extraction culture fluid was extracted twice with a mixture of butanol (3:1). The extracts were combined and centrifuged at 4000 rpm for 15 min. The supernatant was fully evaporated under vacuum. The residue was dissolved in 80 ml of methanol and dichloromethane (1:1) mixture and then filtered with 0.45 µm of polytetrafluoroethylene (PTFE) membrane. The resulting extract was further evaporated in 5 ml of an 85:15 mixture of dichloromethane and methanol. The samples were transferred to a silica gel column (300 mm × 25 mm, 60 A, 35–75 mm). The column was washed to discard the acetone by using MeOH–H₂O (85:15). Finally, the effluent was collected and evaporated to dryness under vacuum. The residue containing the peptides was dissolved in 2 ml of MeOH–H₂O (85:15), transferred to a 1.5 ml centrifuge tube, and again evaporated to dryness vacuum for 10 min. The final residue of peptides was quantified and dissolved in 5 ml of MeOH–H₂O (85:15) for the further analysis.

UPLC-QTOF-MS/MS Analysis of *Trichoderma* Peptaibols

Aliquots of peptaibols (1 µg of peptaibols /2 ml of MeOH) were analyzed using UPLC-QTOF-MS/MS (UMS, ACQUITYTM UPLC & Q-TOF MS Premier). The MS analysis was performed using a turbo data-dependent scan under the same conditions as used for negative-mode MS scanning. Total current ion mass spectra (full-scan mode) were measured between 200 and 2000 m/z. The mixture used for automatic mass calibration was 200 ng mL⁻¹ of leucine enkephalin (556.3 m/z). The resulting 3-D matrix containing arbitrarily assigned peak index (retention time-m/z pairs), sample names (observations), and normalized peak area were exported to SIMCA-P software 11.0 (Umetrics, Umea, Sweden) for multivariate statistical analysis [18–20].

Extraction and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Secondary Metabolites

Secondary metabolites were extracted from culture filtrates of *Trichoderma* as previously described [10]. The selected *Trichoderma* (spore suspension 4.7 × 10³ CFU/ml) was cultured in one liter of a mineral medium containing (g l⁻¹ of 50 % seawater) peptone (10), glucose (5), MgSO₄, NH₄NO₂ (2.4), yeasts extracts (5), ZnSO₄ (0.2), FeSO₄ (0.2) at pH 7.2 and incubated at 28 °C with shaking at 180 rpm for 31 days. After the incubation, 250 ml of

dichloromethane was incorporated in the culture for overnight, and then impurities were removed by filtration (Whatman No.4) Further the supernatant was collected and stored at 2 °C for 24 h, and then the solvent and water were separated by using a separating funnel. The dichloromethane phase washed with the distilled water for two times and then concentrated by a using rotary evaporator. The concentrated residue of 10 µg were dissolved in 100 µl of methanol, passed through a 0.45 µm disposable PTFE filter, and then used for GC-MS (AutoSystem XLGC/TurboMass MS) analysis. To qualitatively analyze the chemical composition of the extracts, GC-MS was used in combination with computer retrieval technology. The normalized gas chromatographic peak area was used to evaluate the relative content of each component.

In Vivo Antagonistic Activity

In order to confirm the antagonistic potential of a selected *T. asperellum* strain ZJSX5003 from previous experiments based on its inhibition rate an in planta antagonistic experiment against FG were performed in greenhouse. For the in planta greenhouse experiment, maize seeds were surface sterilized in 2 % NaClO (Sodium hypochloride) for 3 min and 75 % ethanol for 2 min and then rinsed 3 times in sterile water. The seeds were then allowed to germinate on sterile wet filter paper in 9 cm at 25 °C for 48 h. and shown pots containing 4 kg sterilized loamy and clay nature of native agriculture soil.

The conidia and mycelia fragments of *T. asperellum* strain ZJSX5003 were harvested from the surface of 7-day-old PDA culture and concentrated to approximately 1 × 10⁶ conidia/ml by centrifugation. The following two treatments were maintained: (1) CK (soil inoculated with FG alone); and, (2) T1 (Soil inoculated with *T. asperellum* strain ZJSX5003 and FG). Two days before sowing the germinated maize seeds, the soil was inoculated with FG at a rate of 5 g of FG biomass/kg of soil. Subsequently, the soil was amended with 20 ml spore suspension of *T. asperellum* strain ZJSX5003 per pot and disease incidence was recorded 7 days after *T. asperellum* strain ZJSX5003 treatment according to corn stalk rot (CSR) classification standards [21]. The evaluation of disease was based on a scale for leaf spot disease from grade zero to grade five. Grade zero: no disease spot; Grade 1: no more than 10 %; Grade 2: 11–30 %; Grade 3: 31–50 %; Grade 4: 51–70 %; and Grade 5: more than 70 % and the disease index calculation with the following formula:

Disease index = Σ (number of plants in each disease stage × levels value)/(total number of plants × the highest levels × 100).

The disease reduction was computed with the following formula:

Disease reduction = the disease index of CK—disease index after treatment of *T. asperellum* strain ZJSX5003.

Statistical Analysis

SPSS version 20.0 statistical software package (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) was used for principal component analysis (PCA) for peptaibols screening and discriminant analysis for influencing factors for antagonist assay in which 13 variables were included (Table 2). Main component was established by multiple linear regression equation based on eigenvalues. Each eigenvalue represents the amount of standardized variance that was captured by a single component. In order to assess the significance between *Trichoderma* species, a ANOVA test (one way classifications) with Duncan Post hoc multiple comparison was used on antagonistic and enzyme activity experiments.

Results

In Vitro Antagonistic Activity of *T. asperellum* Isolates

The eight *Trichoderma* strains (including one positive control) exhibited different inhibition rates against FG (Fig. 1a). The maximum inhibition was recorded with strain ZJSX5003 which was 0.48 % higher compared to positive control strain SH2303 (Fig. 1a, b).

Cell Wall-Degrading Enzyme Activity

Cell wall-degrading enzyme (CWDEs) activity was measured in eight strains of *Trichoderma* including the positive control. CWDEs such as chitinase, β -1,3-glucanase, and protease activity significantly varied between the tested strains (Fig. 2). The chitinase activity was generally less compared to positive control (strain SG3403) excepting in the case of the ZJSX5003 test strain, in which higher (4 U/L). β -1,3-glucanase activity was 26 % higher in *T. asperellum* ZJSX5003 than in the positive control (strain SG3403) whereas the protease activity was 47 % higher in *T. asperellum* ZJSX5003.

Identification of Peptaibols and Secondary Metabolites in *Trichoderma*

A total of 30 peptaibiotics were detected in eight *Trichoderma* culture filtrate using UPLC-Q-TOF-MS (Supplementary Table 2) and seven classes of secondary metabolites were identified through GC-MS (Table 1; Supplementary Table 3). The kinds of peptaibols and

secondary metabolites varied between different *Trichoderma* spp. The ZJSX5003 and GDZQ1008 strains produced more than seven kinds of peptaibols compared with other *T. asperellum* strains. The enzymes, peptaibol and secondary metabolites data were mean-centered with unit variance scaling for statistical analysis. Discriminant analysis was performed to observe the correlation of peptaibols, CWDEs and secondary metabolites in relation to antagonistic activity of the *Trichoderma* strains.

Discriminant Analysis

In order to assess the correlation between the *Trichoderma* derived CWDEs, peptaibols, and secondary metabolites in relation to in vitro inhibitory rates (%) against pathogens FG. 13 relevant variables were subjected to discriminant (Table 2). The five most important components explained nearly 90 % of the variation in the original 13 variables. The screen plot determined the optimal number of components as five. Thus, the first five components were used in the linear regression model.

The five factors were selected were chitinase activity, β -1,3-glucanase activity, extracellular protease activity, peptaibol quantity, and polyketide quantity and these were selected for a linear regression model. The first three components accounted for 38.1, 19.8, 13.0 % of the variance, totaling 70.9 %. In other words, chitinase, β -1,3-glucanase and extracellular protease activity could influence the biocontrol results in this study. Orthogonal vectors and the eigenvector matrix were standardized and the five principal component scores were obtained based on the standardized orthogonal feature matrix. The relationship between biocontrol potential and the five most important variables was arrived at with the following linear regression model equation:

$$Y = 0.3810y_1 + 0.19082y_2 + 0.13039y_3 + 0.11192y_4 + 0.07241y_5 \quad (1)$$

where Y is the integrated evaluation value of the synthesized five principal components and y is the principal component score after standardization.

Based on this equation, *T. asperellum* ZJSX5003 exhibited the highest correlation value (1.266), followed by CDZQ1008 (0.470) and GDFS1009 (0.213) (Table 3). The positive control *T. harzianum* SH2303 had an average value of 0.572.

Among these strains, *T. asperellum* ZJSX5003, GDFS1009, and GDZQ1008 were observed far from the midline axis and showed significant antagonistic and diversified peptaibols ($p < 0.05$; Fig. 3a, b; Table 3). The three *Trichoderma* strains contained significantly different types of peptaibols ($p < 0.01$) as follows: Trichovirin-Ib in

Fig. 1 **a** In vitro antagonistic activity of different *T. asperellum* strains and positive control of *T. harzianum* SH2303 against *F. graminearum*. Results shown are mean $\pm$ SEM ($n = 3$), bars with a same letters are not statistically different among the antagonistic activity of *Trichoderma* strains following Duncan's test ($p < 0.05$). **b** In vitro antagonist activity; **a** *Fusarium graminearum* on PDA petri dish, **b** antagonism of *T. asperellum* strain ZJSX5003 (T) against *Fusarium graminearum* (FG)

Fig. 2 Cell wall-degrading enzyme activity of *Trichoderma* strains, results shown are mean $\pm$ SEM ($n = 3$), bars with a same letters are not statistically different among the enzyme activity of *Trichoderma* strains following Duncan's test ($p < 0.05$)

Table 1 Secondary metabolites and their concentration produced by *Trichoderma* strains were analyzed by gas chromatography-mass spectrometry (GC-MS)

<i>Trichoderma</i> strains	Content of antibiosis secondary metabolites (%)						
	Polyketides	Terpenes	CAD	NHC	Alkanes	Ethanols	Aldehydes
SH2303	3.42 ± 0.2 ^d	11.82 ± 1.5 ^d	15.04 ± 1.2 ^a	1.99 ± 0.5 ^a	34.96 ± 2.3 ^d	2.59 ± 1.2 ^c	0.00 ± 0.0 ^a
ZJSX5003	3.84 ± 0.8 ^d	22.18 ± 3.2 ^d	18.42 ± 5.6 ^a	2.97 ± 0.8 ^b	21.18 ± 1.5 ^c	0.05 ± 0.0 ^a	0.18 ± 0.1 ^a
GDFS1009	1.15 ± 0.5 ^b	0.00 ± 0.0 ^a	45.09 ± 8.9 ^c	14.40 ± 1.4	15.30 ± 3.2 ^b	0.21 ± 0.1 ^a	0.23 ± 0.1 ^a
ZJSX5002	2.20 ± 0.9 ^c	2.22 ± 0.8 ^a	18.55 ± 4.5 ^a	2.41 ± 0.5 ^b	16.38 ± 1.5 ^b	2.42 ± 0.5 ^c	14.49 ± 2.5 ^c
HNLY1002	0.40 ± 0.1 ^a	1.43 ± 0.6 ^a	37.18 ± 6.5 ^c	6.41 ± 2.5 ^c	22.46 ± 2.3 ^c	0.22 ± 0.1 ^a	1.81 ± 0.5 ^b
HNCS4002	6.01 ± 1.5 ^e	4.05 ± 1.2 ^b	13.09 ± 2.5 ^a	7.93 ± 1.5 ^d	21.05 ± 6.2 ^c	3.44 ± 1.2 ^d	1.86 ± 0.6 ^b
GDZQ1008	3.75 ± 0.9 ^d	9.87 ± 2.5 ^c	28.79 ± 8.5 ^b	5.36 ± 1.2 ^c	25.86 ± 1.4 ^c	1.30 ± 0.9 ^b	0.69 ± 0.2 ^a
GDFS5001	5.28 ± 0.5 ^e	1.94 ± 0.8 ^a	60.14 ± 7.2 ^d	3.98 ± 1.2 ^{bc}	5.98 ± 3.2 ^a	0.16 ± 0.1 ^a	1.59 ± 0.8 ^b

The results showed mean value ± standard error (n = 3), one way ANOVA followed by multiple comparison of Duncan test

CAD carboxylic acids and derivatives, NHC nitrogen heterocyclic compounds; The different alphabets in the superscript differ significantly ($p < 0.05$) between the *Trichoderma* strains

T. asperellum ZJSX5003, Trichotoxin_A-50_F in *T. asperellum* GDFS1009, and Trichorzin_HA_III, Trichotoxin_A-40, and Hypomurocin_B_IV in *T. asperellum* GDZQ1008. Therefore, a total of five peptaibols are the potential factors that influenced the biocontrol activity of *Trichoderma*.

Polyketides and terpenes showed a positive correlation with antagonism of *Trichoderma*. Polyketones (i.e. pyrones, 6PP) and terpenes with broad and high antagonizing activity were recorded as ZJSX5003 (26 %), SG3403 (17 %), SG2303 (15 %), and GDZQ1008 (14 %). Carboxylic acids and their derivatives were abundant in strain ZJSX5003 (18 %). Thus, the strain ZJSX5003 seemed to be better at antagonism and growth promotion.

This model predicted results were consistent with the laboratory experimental results of in vitro antagonistic activity and enzyme activity, in addition the greenhouse experiments also confirmed the strain ZJSX5003 as potent to control FG a casual agent of CSR in maize with disease reduction of 71 % (Fig. 4).

Discussion

The biological control of corn stalk rot (CSR) in maize crops would greatly increase the production of corn worldwide. Several control strategies, such as the selection of resistant corn varieties, improved cultivation techniques, seed coating treatments, and use of biological control agents have been attempted. The seed coating is not effective against CSR due to its non-lasting preventative effects after the seedling stage. However, Several *Trichoderma* agents are commercially available as BCAs against CSR but their effects, virulence and mechanisms are not

clear. Therefore, in this study, the antagonistic activity of eight *T. asperellum* strains was compared for the selecting the effective antagonist stain against CSR caused by FG.

The main factors such as hydrolytic enzymes (chitinase, β -1,3-glucanase, protease) and secondary metabolites (peptaibols and polyketides) significantly contributed to antagonistic activity of *Trichoderma* against FG. The components of antagonistic function of the *Trichoderma* could be due to the effects of chitinase, β -1,3-glucanase, protease, peptaibols, and polyketides. Mycoparasitism is usually mediated by a set of CWDEs and these also seem to play a role in *Trichoderma*'s antagonistic effects against *Fusarium* [22, 23]. Comparative genomic studies revealed that *Trichoderma* evolved from a mycoparasitic ancestor [24, 25] Six out of 30 *T. asperellum* isolates showed high antagonistic activity against *Fusarium oxysporum* f. sp. *lycopersici* isolates due to production of high amounts of CWDEs.

Trichoderma spp., have an active metabolism and they can produce large amounts of enzymes that act as potent weapons against other non beneficial fungi [26]. *Trichoderma* spp., also secrete a chemically diverse range of secondary metabolites, including peptaibols, polyketides, pyrones, terpenes, and polypeptides [27, 28]. A previous study reported on the significance of secondary metabolites in the antagonistic action of *Trichoderma* spp., against the pathogenic fungi *Pythium ultimum* and *Rhizoctonia solani* [10, 29]. We also found that *Trichoderma* produced a diverse group of metabolites that were species-specific. The antagonistic effects of *T. asperellum* isolate ZJSX5003 were positively correlated with their production of peptaibols and polyketides. Similarly, *T. asperellum* produced rich volatile and secondary metabolites which are effective biocontrol agents against pathogens such as *F. oxysporum*, *R. solani* and

Table 2 The selected list of antagonistic activity influencing factors of *Trichoderma* against pathogen: *F. graminearum* Schw for the statistical evaluation study

Factors	Code	<i>Trichoderma</i> strains							
		SH2303	GDFS1009	ZJSX5003	ZJSX5002	HNLY1002	HNCS4002	GDZQ1008	GDFS5001
Chitinase activity (U)	x1	4.14 ± 1.2 ^d	3.61 ± 0.2 ^c	4.07 ± 0.5 ^d	3.72 ± 1.2 ^c	2.44 ± 1.5 ^b	2.84 ± 1.2 ^b	2.89 ± 1.2 ^b	1.20 ± 0.2 ^a
β-1,3-glucanase activity (U)	x2	0.59 ± 0.2 ^b	0.6 ± 0.3 ^c	0.80 ± 0.2 ^d	0.50 ± 0.5 ^b	0.66 ± 0.2 ^c	0.72 ± 0.5 ^d	0.45 ± 0.3 ^a	0.62 ± 0.3 ^c
Protease activity (U)	x3	2.48 ± 0.9 ^b	2.48 ± 0.8 ^b	4.85 ± 0.8 ^d	1.72 ± 0.5 ^a	3.13 ± 1.2 ^c	2.6485 ± 0.2 ^b	3.8107 ± 0.5 ^c	2.38 ± 0.8 ^b
Peptaibol number	x4	2 ± 0.2 ^a	6 ± 1.5 ^c	7 ± 1.4 ^{cd}	4 ± 1.5 ^b	3 ± 0.8 ^b	1 ± 0.6 ^a	9 ± 2.4 ^d	1 ± 0.1 ^a
Polyketides (relative %)	x5	2.40 ± 0.5 ^b	1.15 ± 0.6 ^a	3.84 ± 1.5 ^c	2.20 ± 1.2 ^b	0.40 ± 0.1 ^a	6.01 ± 1.5 ^d	3.75 ± 1.6 ^c	5.28 ± 1.2 ^{cd}
Terpenes (relative %)	x6	14.53 ± 0.6 ^{cd}	0.00 ± 0.0 ^a	22.18 ± 4.6 ^d	2.22 ± 0.5 ^a	1.43 ± 0.6 ^a	4.05 ± 2.4 ^b	9.87 ± 1.4 ^c	1.94 ± 0.7 ^a
Alkane-including hydrocarbon (%)	x7	0.00 ± 0.0 ^a	15.30 ± 1.2 ^c	21.18 ± 2.5 ^d	16.38 ± 2.6 ^{cd}	22.46 ± 2.5 ^d	0.00 ± 0.0 ^a	25.86 ± 3.5 ^d	5.98 ± 0.9 ^b
Carboxylic acids and derivatives	x8	17.34 ± 1.5 ^b	18.42 ± 1.5 ^b	45.09 ± 5.6 ^d	18.55 ± 1.5 ^b	37.18 ± 4.5 ^c	13.09 ± 1.5 ^a	28.79 ± 1.8 ^{cd}	60.14 ± 4.2 ^e
Aldehyde-including hydrocarbon (%)	x9	0.22 ± 0.1 ^a	0.23 ± 0.1 ^a	0.18 ± 0.1 ^a	14.49 ± 2.5 ^c	1.81 ± 0.6 ^b	1.86 ± 0.6 ^b	0.69 ± 0.5 ^a	1.59 ± 0.6 ^b
Nitrogen heterocyclic compounds	x10	2.02 ± 0.2 ^a	14.40 ± 1.2 ^d	2.97 ± 1.5 ^a	2.41 ± 0.8 ^a	6.41 ± 1.5	7.93 ± 2.4 ^{cd}	5.36 ± 1.4 ^c	3.98 ± 1.2 ^b
Alcohols (%)	x11	0.00 ± 0.0 ^a	0.21 ± 0.1 ^b	0.05 ± 0.0 ^a	2.42 ± 0.9 ^{cd}	0.22 ± 0.1 ^b	3.44 ± 1.6 ^d	1.30 ± 0.8 ^c	0.16 ± 0.05 ^b
Inhibitory rate of pathogen in vitro (%)	x12	74.12 ± 5.6 ^d	68.24 ± 6.5 ^b	74.48 ± 3.5 ^d	66.10 ± 5.8 ^b	71.3 ± 42.6 ^c	65.85 ± 5.6 ^b	65.09 ± 5.2 ^b	43.53 ± 5.4 ^a
Antagonistic effect of pathogen (%)	x13	64.28 ± 2.6 ^d	66.67 ± 7.5 ^d	70.67 ± 2.5 ^e	48 ± 4.5 ^b	55.11 ± 4.5 ^c	55.11 ± 4.5 ^c	63.11 ± 4.9 ^d	16.18 ± 1.9 ^a

The results showed mean value ± standard error (n = 3), one way ANOVA followed by multiple comparison of Duncan test

The different alphabets in the superscript differ significantly ($p < 0.05$) between the *Trichoderma* strains

Table 3 Principal components analysis and comprehensive scores

<i>Trichoderma</i>	y ₁	y ₂	y ₃	y ₄	y ₅	Y
SH2303	1.379	−0.396	1.550	−0.981	0.446	0.572
GDFS1009	1.349	−0.505	−2.639	1.147	0.208	0.213
ZJSX5003	1.193	3.583	0.769	0.197	−0.279	1.266
ZJSX5002	0.687	−2.707	0.212	−1.628	−0.706	−0.480
HNLY1002	0.040	0.279	−1.670	−0.093	−0.527	−0.196
HNCS4002	−0.294	−1.487	1.357	2.333	1.257	0.123
GDZQ1008	1.063	0.862	−0.405	−1.469	1.536	0.470
GDFS5001	−6.186	0.406	−0.092	−0.363	−0.024	−2.331

Fig. 3 A partial least-squares discriminant analysis (PLS-DA) model of the UPLC-QTOF-MS spectral data of peptaibols present in different *Trichoderma* strains, **a** the scores plot of differential *Trichoderma* strains based on analysis differential peptaibols spots, the horizontal and vertical axis indicate differences between groups and within groups, **b** the loading plot show the correlation analysis of which peptaibols are the major components that determine the difference in *Trichoderma* strains

P. ultimum [26]. Each compound exhibits a specific antibiotic activity and each *Trichoderma* strain has different antagonistic effect on different pathogens. Secondary metabolites serve a pivotal function in antagonistic activities against *Fusarium* and also act as sporogenic factors and growth promoters that affect morphological differentiation

in mycoparasitic *Trichoderma* [30, 31]. In view of this specific secondary metabolites could be of a commercially viable alternative for controlling phytopathogens rather than whole organism formulations [31].

The challenge remains to determine which secondary metabolites would be most effective in mitigating the

Fig. 4 Effect of *T. asperellum* ZJSX5003 treatment on biological control of CSR caused by FG, CK (Soil inoculated with FG alone), T1 Soil inoculated with *T. asperellum* strain ZJSX5003 and FG)

effects of *Fusarium* and how they can be applied to corn seedlings for a sustainable effect. Differential antagonism activity has been observed for *Trichoderma* isolates belonging to the same species, as shown by a semi-specificity in the interaction of *Trichoderma* with spores of *Botrytis cinerea* and *M. phaseolina* [32, 33]. Testing for the strain of *Trichoderma* with the highest antagonistic activity is important for developing biocontrol agents. A correlation result of this study indicated that hydrolytic enzyme and some metabolites played a key role in controlling CSR. In particular, chitinase was the most highly correlated hydrolytic enzyme, emphasizing its relevance for biocontrol of FG. This result is consistent with the hypothesis that *Trichoderma* sp., are able to repress pathogen through synchronization of mycoparasitism and antibiotic production [22, 34, 35].

Finally, we detected 70 different chemical compounds in *T. asperellum* ZJSX5003. The isolates produced more secondary metabolites than other strains tested including alkanes (21 %), terpenes (22 %), carboxylic acids and derivatives (18 %), and others. These metabolites promote the transfer of soil insoluble elements, such as phosphorus, iron, and manganese into soluble nutrients available for plants, and promote plant growth and also increase immunity. Our in vivo greenhouse test confirmed that *T. asperellum* ZJSX5003 is an effective potential strain against FG.

Conclusion

Integration of the antagonistic assay in vivo and in vitro and the metabolic data gathered suggested that *T. asperellum* ZJSX5003 had the best antagonistic activity and was the most promising antagonist against the

pathogen FG. This study lays the foundation for using *T. asperellum* ZJSX5003 as a biocontrol of CSR in field situations paving the way for the use of this strain in understanding the molecular basis of fungal antagonism and their exploitation in improving crop production.

Acknowledgments We thank the financial support from the Special Project of Basic Work for Science and Technology (2014FY120900), the key project of the basic research of Shanghai Municipal Science and Technology Commission (12JC1404600), Natural Science Foundation of China (No. 31201557), Natural Science Fund of Shanghai (No.12ZR1414100), the special fund of Modern Agricultural Industry Technology System (CARS-02), Ministry of Education University Doctoral Foundation (No. 20120073120070), and the SJTU Medical-Engineering Cross Research Fund (No. YG2015MS37).

Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

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