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ORIGINAL ARTICLE

Metabolic profiling of *Fusarium oxysporum* f. sp. *conglutinans* race 2 in dual cultures with biocontrol agents *Bacillus amyloliquefaciens*, *Pseudomonas aeruginosa*, and *Trichoderma harzianum*

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Abstract

There are increasing efforts to identify biocontrol-active microbial metabolites in order to improve strategies for biocontrol of phytopathogens. In this work, *Fusarium oxysporum* f. sp. *conglutinans* was confronted with three different biocontrol agents: *Trichoderma harzianum*, *Bacillus amyloliquefaciens*, and *Pseudomonas aeruginosa* in dual culture bioassays. Metabolites produced during the microbial interactions were screened by a matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). *T. harzianum* exhibited the strongest inhibition of growth of *F. oxysporum* resulting in overlay of the pathogen colony with its mycelium. Recorded metabolite profiles suggested a direct attack of *F. oxysporum* mycelium by *T. harzianum* and *B. amyloliquefaciens* by means of membrane-attacking peptaibols and a set of antimicrobial lipopeptides and siderophores, respectively. The direct mode of the biocontrol activity of *T. harzianum* and *B. amyloliquefaciens* corresponded to their ability to suppress *F. oxysporum* production of mycotoxin beauvericin suggesting that this ability is not specific only for *Trichoderma* species. In the case of *P. aeruginosa*, siderophores pyoverdine E/D and two rhamnolipids were produced as major bacterial metabolites; the rhamnolipid production was blocked by *F. oxysporum*. The results showed that this type of biocontrol activity was the least effective against *F. oxysporum*. The effective application of MALDI-MS profiling to the screening of nonvolatile microbial metabolites produced during the interaction of the phytopathogen and the biocontrol microorganisms was demonstrated.

Introduction

The biological control of fungal phytopathogens by microbes is a widely studied strategy. However, the mechanisms through which antagonistic microorganisms affect pathogens are often not clear. Members of *Fusarium oxysporum* species complex are typically ubiquitous soil-borne pathogens that cause vascular wilt and root rot in a wide range of plants and are considered among top 10 fungal pathogens in molecular plant pathology based on the scientific and economic importance (Dean et al. 2012; Aoki et al. 2014). *F. oxysporum* f. sp. *conglutinans* (Foc) causes fusarium wilt of cabbage that is responsible for significant economic losses (Li et al. 2015a; Liu et al. 2017).

Bacterial lipopeptides, fungal sesquiterpenoids, and volatile terpenes have been recognized to play important roles in microbial interactions and biological control (Romero et al. 2007; Malmierca et al. 2016; Schmidt et al. 2016). Pyrrolnitrin and pyoverdine were demonstrated to participate in pseudomonas biocontrol activities (Paulitz and Loper 1991; Ligon et al. 2000). *B. amyloliquefaciens* produced surfactin, iturin, and fengycin in the interaction zone with the pathogen *Macrophomina phaseolina* (Torres et al. 2016). In *Trichoderma* species, a variety of biocontrol-active metabolites was described (Liu et al. 2016; Mutawila et al. 2016; Pascale et al. 2017), including an active production of siderophores (Angel et al. 2016).

Modern metabolomic analyses offer better means for identification of broad profiles of metabolites compared to previous approaches. They have been used to decipher the host resistance mechanisms against fungal pathogens (e.g., Gunnaiah and Kushalappa 2014; Dhokane et al.

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2016; Li et al. 2017). This work focused on profiling microbial metabolites produced during growth of the fungal pathogen *F. oxysporum* f. sp. *conglutinans* race 2 with three biocontrol agents (BCAs), *P. aeruginosa*, *B. amyloliquefaciens*, and *T. harzianum*, in dual cultures on the agar medium using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). The Foc race 2 strain was used because of its much higher pathogenicity compared to race 1 (Li et al. 2015b). The microbes were grown in dual cultures on agar media and metabolites present in the Foc-BCA interaction zones were analyzed.

Materials and methods

Microorganisms

Fusarium oxysporum f. sp. *conglutinans* race 2 strain (Foc) was obtained from the Isolate Collection of Naktuinbouw (Roelofarendsveen, The Netherlands) and *Trichoderma harzianum* CCF2714 from the Culture Collection of Fungi (Charles University, Prague, Czech Republic). Fungal strains were maintained on potato dextrose agar (PDA, Oxoid Ltd., UK–potato extract 4 g/L, dextrose 20 g/L, agar 15 g/L, pH 5.6) and stored at 4 °C. *Bacillus amyloliquefaciens* DSM23117 was obtained from DSMZ–German Collection of Microorganisms and Cell Cultures (Germany) and *Pseudomonas aeruginosa* ATCC 15692 from American Type Culture Collection (USA). Bacteria were stored in a Luria-Bertani medium (LB; consisting of tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, pH 7.0) containing 30% glycerol at − 80 °C.

Instruments and chemicals

MALDI-MS experiments were performed on a 12T SolariX FTICR (Fourier transform ion cyclotron resonance) mass spectrometer (Bruker Daltonics, Billerica, MA, USA). Full-scan MS data were acquired in a mass range 40–3000 *m*/*z* with an external calibration on a mixture of peptides (Pepmix II, Bruker Daltonics, Germany) and clusters of matrices with a mass accuracy better than 5 ppm. Samples (2 μL) were spotted on MALDI plate, dried, and covered with either α-cyano-4-hydroxycinnamic acid (CHCA) [2 μL of CHCA, 10 mg/mL in 50% acetonitrile (ACN)─0.1% trifluoroacetic acid] or 2,5-dihydroxybenzoic acid (2 μL of DHB, 10 mg/mL in 50% ACN─0.1% trifluoroacetic acid) matrix. Instrument parameters were optimized and desorption of the samples was performed with SmartBeam II laser (laser power 30%, 200 scans, 2 kHz). MALDI matrices were obtained from Bruker Daltonics (Germany). The high-purity solvents used for MALDI-MS analysis were obtained from Sigma-Aldrich (USA) and Merck (Germany).

Dual culture assays

Fungal strains were inoculated with a mycelial plug (7 mm in diameter) cut from a stock culture and the bacteria with an amount of 3×10^7 bacterial cells (volume 10 μL) grown overnight in the liquid LB medium at 30 °C and 160 rpm. All cultures were incubated at 28 °C for 10 days. The experiments were conducted in triplicates.

Metabolic profiling

For metabolomic studies, the dual cultures and individual strains were grown on the Bushnel-Haas agar medium (BH; consisting of magnesium sulfate 0.2 g/L, calcium chloride 0.02 g/L, potassium hydrogenphosphate 1 g/L, potassium dihydrogenphosphate 1 g/L, ammonium nitrate 1 g/L, ferric chloride 0.05 g/L, glucose 5 g/L, and agar 20 g/L, pH 7.0). Fe^{3+} ions were thus a component of all metabolomic studies. The metabolites produced by the microorganisms were determined after a 5-day cultivation, and the samples were taken from control cultures of the individual microbes and from the interaction zones of dual cultures. To determine the metabolites, samples of 1 g of BH agar from the individual strain cultures and the interaction zones of dual cultures were cut and homogenized by pressing through a syringe. The homogenized agar was centrifuged at 16000*g* for 10 min. The supernatants were transferred into clean tubes and stored at − 80 °C.

MALDI-MS and MS/MS spectra were acquired in positive-ion mode in the range of 150–3000 Da. The spectrum of fusaric acid was acquired in the range of 40–1500 Da. Samples were mixed with matrix (CHCA or DHB) in 1:1 ratio, the mixture was spotted on a MALDI plate, and the mass spectra were collected. The mass spectra were searched using CycloBranch software (Novák et al. 2015, 2017) against in-house metabolite databases designed for all studied BCAs. The isolated ions were further subjected to analysis by tandem mass spectrometry (MS/MS) and the resulting spectra were compared to the spectra in a public GNPS Library [http://gnps.ucsd.edu/, spectra no. CCMSLIB00000846455 (fusaric acid), CCMSLIB00003739997 (bacillibactin), CCMSLIB00000006851 (3-O-(α-L-rhamnopyranosyl-(1–2)-α-L-rhamnopyranosyl)-3-hydroxydecanoyl-3-hydroxydecanoic acid), CCMSLIB00003142432 (beauvericin)] or compared to "in silico" generated MS/MS spectra of the corresponding metabolites by CycloBranch. The minimum threshold of relative intensity and *m*/*z* error tolerance were 1% and 5 ppm, respectively.

Results and discussion

Dual culture assays on PDA medium

In dual cultures with Foc *T. harzianum* was able to inhibit the growth of Foc starting from day 2 of cultivation on PDA medium (Fig. 1). In later stages, *T. harzianum* overlaid the Foc colony with its mycelium and a red metabolite appeared beneath the colony (Fig. 1b). Production of red and purple polyketide and naphthoquinonic pigments by soil-borne phytopathogenic strain of *F. oxysporum* have been described, the former having antifungal properties (Limon et al. 2010; Lebeau et al. 2018). Both bacterial strains exhibited a partial inhibition of Foc growth with a clear inhibition zone of 2–5 mm formed between Foc and *B. amyloliquefaciens* (Fig. 1).

The differences in Foc inhibition by individual BCA could be attributed to different biocontrol mechanisms. *Trichoderma* species are known to influence mycelial growth of *Fusarium* species in dual culture bioassays on PDA medium (Blaszczyk et al. 2017; Sharma et al. 2017). *T. harzianum* T-soybean has been recently shown to inhibit growth of Foc by parasitic function (Zhang et al. 2017). *B. amyloliquefaciens* is supposed to inhibit pathogenic fungi due to fungicidal effects of its lipopeptides as demonstrated in dual cultures with *Macrophomina phaseolina* (Torres et al. 2016), while in the case of pseudomonads, the biocontrol activity towards Foc was connected with the production of siderophore pseudobactin B10 (Kloepper et al. 1980).

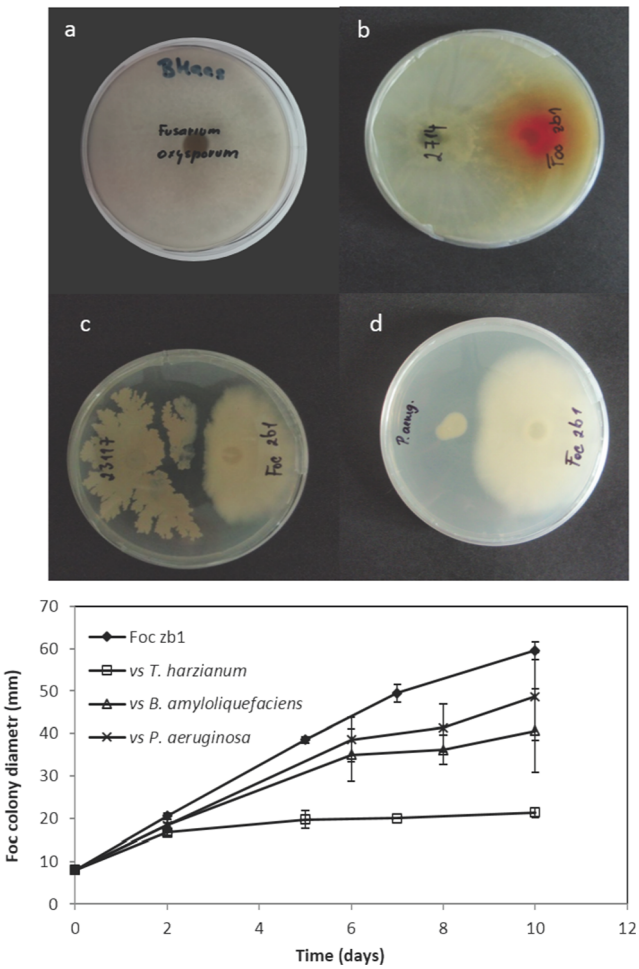

Fig. 1 Growth of *Fusarium oxysporum* f. sp. *conglutinans* (Foc zb1) in co-cultivation with biocontrol agents on PDA medium. Figures of 10-day old cultures: **a** control culture of Foc; **b** Foc-*T. harzianum* dual culture; **c** Foc-*B. amyloliquefaciens* dual culture; **d** Foc-*P. aeruginosa* dual culture; **e** Foc colony diameter after 10-day growth

Metabolic profiling

In order to analyze microbial metabolites, the cultures were grown on mineral BH medium to avoid the interference of complex media constituents with the analyses. In this experiment, the inhibition measured in dual cultures was similar to that recorded on PDA (data not shown), except that no red colored metabolite was visible in Foc-*T. harzianum* co-cultures. The metabolites identified in the control cultures of individual microbes are shown in Table 1. MS/MS spectra of the individual compounds are reported in Online Resource (Figs. S2-S11).

In single Foc cultures, a limited number of metabolites were detected. Unlike *F. oxysporum* strain FGSC9935 that has recently been reported to produce three different ferrichrome siderophores (Lopez-Berges et al. 2012), ferricrocin was the only detected siderophore in Foc cultures in this work. Ferricrocin was also the only siderophore measured in *T. harzianum* control cultures although up to 15 siderophores were reported to be produced by this fungus (Lehner et al. 2013). Those observations can probably be explained by the presence of Fe^{3+} ions in the medium during metabolomic studies. Further, the mycotoxin beauvericin was detected in MALDI-MS spectra of Foc cultures. As no enniatins were found, the Foc strain probably belongs to *Fusarium* species with a beauvericin-producing profile (Liuzzi et al. 2017). Production of fusaric acid was also recorded in Foc cultures (Table 1). This toxin is responsible for phytotoxic effects of Foc on tomato plants (Singh et al. 2017) and may help the fungus compete with other microbes in soil (Martin-Rodriguez et al. 2014; Quecine et al. 2016).

In the control cultures of *P. aeruginosa* (Table 1), the siderophores pyoverdine E/D were produced that could help compete with the siderophore activity of fusaric acid produced by Foc (cf. Ruiz et al. 2015). The bacterium also produced rhamnolipids that are known to exhibit antifungal effects (Reddy et al. 2016; DaSilva Araujo et al. 2017).

In the control cultures *of B. amyloliquefaciens*, fengycin A and bacillomycin D1 and D2 were detected (Table 1). Three molecules of fengycin A with different lengths of C chains were found. Fengycin and iturin lipopeptides as well as bacillomycins D1/D2 are known to have antifungal activities

Table 1 Metabolites identified in control cultures of individual microbes

Organism	Metabolite	Ion type	Measured *m/z*	Mass spectrum
Fusarium oxysporum f. sp. *conglutinans* (Foc)	Beauvericin	$[M + Na]^+$	806.399	Fig. S5
		$[M + K]^+$	822.373	
	Ferricrocin	$[M + Na]^+$	740.318	Fig. S4
		$[M + K]^+$	756.292	
	Fusaric acid	$[M + H]^+$	180.102	Fig. S3
Pseudomonas aeruginosa	3-O-α-L-rhamnopyranosyl-3-hydroxydecanoyl-3-hydroxydecanoic acid	$[M + Na]^+$	527.319	Fig. S10
		$[M + K]^+$	543.293	
	3-O-(α-L-rhamnopyranosyl-(1-2)-α-L-rhamnopyranosyl)-3-hydroxydecanoyl-3-hydroxydecanoic acid	$[M + Na]^+$	673.377	Fig. S9
		$[M + K]^+$	689.351	
	Pyoverdine D	$[M + H]^+$	1334.599	Fig. S11
	Pyoverdine E	$[M + H]^+$	1333.614	Fig. S11
Bacillus amyloliquefaciens	Bacillibactin	$[M + Na]^+$	905.244	
		$[M + K]^+$	921.218	
		$[M + H]^+$	883.263	Fig. S2
	Bacillomycin D1	$[M + H]^+$	1031.541	Fig. S6
		$[M + Na]^+$	1053.523	
		$[M + K]^+$	1069.496	
	Bacillomycin D2	$[M + H]^+$	1045.556	Fig. S7
		$[M + Na]^+$	1067.538	
		$[M + K]^+$	1083.512	
	Fengycin A (C15-OH)	$[M + H]^+$	1449.788	
		$[M + Na]^+$	1471.774	
		$[M + K]^+$	1487.745	
	Fengycin A (C16-OH)	$[M + H]^+$	1463.801	Fig. S8
		$[M + Na]^+$	1485.785	
		$[M + K]^+$	1501.759	
	Fengycin A (C17-OH)	$[M + H]^+$	1477.822	
		$[M + Na]^+$	1499.803	
		$[M + K]^+$	1515.780	
Trichoderma harzianum	Ferricrocin	$[M + Na]^+$	740.318	Fig. S4
		$[M + K]^+$	756.292	
	Peptaibols	see Table 3		Fig. S1

(Caldeira et al. 2011; Gu et al. 2017) and are involved in competitive interactions with other microorganisms (e.g., Torres et al. 2016; Ben Ayed et al. 2017). Bacillibactin, a trilacton-based natural siderophore, was also found in the *B. amyloliquefaciens* monocultures (Table 1). Lipopeptide and siderophore profiles of *B. amyloliquefaciens* detected were in accordance with the reports of *B. amyloliquefaciens* SQR9 that was shown to fine-tune its lipopeptide and bacillibactin production to control different fungal pathogens (Li et al. 2014) (Table 2).

In the control cultures of *T. harzianum*, besides the siderophore ferricrocin mentioned above (Table 1), peptaibols were also detected and their MALDI-MS spectrum is shown in Online Resource (Fig. S1). The presence of 11-, 14-, and 18-residue series of these peptides (Table 3) was confirmed on the basis of the precise mass measurement and a simulation of the molecular formula of each isobaric group of peptides (Mukherjee et al. 2011). Detection of these membrane-attacking, antibiotic peptides suggested a parasitic mode of action of *T. harzianum* in the antagonistic interactions and corroborated the hypothesis that *Trichoderma* species attack phytopathogens by destroying fungal cell walls by a synergistic action of lytic enzymes and antibiotic metabolites (Benitez et al. 2004).

The metabolites detected in co-cultures of Foc and BCAs are summarized in Tables 2 and 4 to show their changes occurring during the antagonistic interactions in these cultures suggesting possible mechanisms involved. Generally, the antagonist interactions involved were operating at two different levels. The first level was that of the iron available in the environment as reflected by the changes in the production of compounds involved in the sequestration of iron. The co-cultivation with both bacteria resulted in disappearance of the production of the intracellular siderophore ferricrocin by Foc that was found to be involved in intracellular iron storage in aspergilli and whose deficiency resulted in a reduced virulence of *A. fumigatus* and *Magnaporthae grisaea* (Hof et al. 2007; Wallner et al. 2009). On the other hand, the production of fusaric acid by Foc also exhibiting the iron sequestration activity (Ruiz et al. 2015) was maintained in co-cultures

Table 2 Metabolites identified in dual cultures of Foc with the individual BCAs

Metabolite	Ion type	Measured *m/z*
Foc/*Pseudomonas aeruginosa*		
Beauvericin	$[M+Na]^+$	806.398
	$[M+K]^+$	822.372
Fusaric acid	$[M+H]^+$	180.102
Pyoverdine D	$[M+H]^+$	1334.597
	$[M+K]^+$	1372.553
Pyoverdine E	$[M+H]^+$	1333.613
	$[M+K]^+$	1371.568
Foc/*Bacillus amyloliquefaciens*		
Bacillibactin	$[M+H]^+$	883.263
	$[M+Na]^+$	905.244
	$[M+K]^+$	921.219
Bacillomycin D1	$[M+H]^+$	1031.541
	$[M+Na]^+$	1053.523
	$[M+K]^+$	1069.497
Bacillomycin D2	$[M+H]^+$	1045.556
	$[M+Na]^+$	1067.538
	$[M+K]^+$	1083.512
Fengycin A (C15-OH)	$[M+H]^+$	1449.788
	$[M+Na]^+$	1471.774
	$[M+K]^+$	1487.745
Fengycin A (C16-OH)	$[M+H]^+$	1463.801
	$[M+Na]^+$	1485.785
	$[M+K]^+$	1501.759
Fengycin A (C17-OH)	$[M+H]^+$	1477.822
	$[M+Na]^+$	1499.803
	$[M+K]^+$	1515.780
Fusaric acid	$[M+H]^+$	180.102
Foc/*Trichoderma harzianum*		
Ferricrocin	$[M+H]^+$	718.336
	$[M+Na]^+$	740.318
	$[M+K]^+$	756.292
Fusaric acid	$[M+H]^+$	180.102
Peptaibols	see Table 3	

Table 3 Peptaibols detected in the extract from *T. harzianum* monoculture

Number of residues	Molecular formula	Ion type	Measured *m/z*
11	$C_{58}H_{102}N_{12}O_{13}$	$[M+Na]^+$	1197.758
		$[M+K]^+$	1213.732
	$C_{59}H_{104}N_{12}O_{13}$	$[M+Na]^+$	1211.774
		$[M+K]^+$	1227.748
	$C_{60}H_{106}N_{12}O_{13}$	$[M+Na]^+$	1225.790
		$[M+K]^+$	1241.764
14	$C_{70}H_{121}N_{15}O_{17}$	$[M+Na]^+$	1466.896
		$[M+K]^+$	1482.870
	$C_{71}H_{123}N_{15}O_{17}$	$[M+Na]^+$	1480.912
		$[M+K]^+$	1496.886
	$C_{72}H_{125}N_{15}O_{17}$	$[M+Na]^+$	1494.928
		$[M+K]^+$	1510.902
18	$C_{81}H_{142}N_{20}O_{22}$	$[M+Na]^+$	1770.051
		$[M+K]^+$	1786.025
	$C_{82}H_{144}N_{20}O_{22}$	$[M+Na]^+$	1784.067
		$[M+K]^+$	1800.041
	$C_{83}H_{146}N_{20}O_{22}$	$[M+Na]^+$	1798.082
		$[M+K]^+$	1814.056

Table 4 Metabolites involved in biocontrol interactions of Foc with bacterial and fungal antagonists

Dual cultures	Metabolites involved in the interaction	Impact of the interaction	Mechanism of biocontrol
Foc/*Pseudomonas aeruginosa*	Beauvericin	Production (by Foc) present	Antibacterial activity (Meca et al. 2010)
	Ferricrocin	Production (by Foc) disappeared	Sequestration of iron (Lopez-Berges et al. 2012)
	Fusaric acid	Production (by Foc) present	Antibacterial activity, sequestration of iron, increase of pyoverdine secretion by the bacterium, quorum sensing inhibitor (Ruiz et al. 2015; Tung et al. 2017)
	Pyoverdine E/D	Production (by bacterium) present	Sequestration of iron (Cezard et al. 2015)
	Rhamnolipids	Production (by bacterium) disappeared	Antifungal activity (Haba et al. 2003)
Foc/*Bacillus amyloliquefaciens*	Bacillomycins D-1/D-2	Production (by bacterium) present	Antifungal/fungicidal activity (Gu et al. 2017)
	Bacillibactin	Production (by bacterium) present	Sequestration of iron (Li et al. 2014)
	Beauvericin	Production (by Foc) disappeared	Antibacterial effect (Meca et al. 2010)
	Fengycin A (C15-C17)	Production (by bacterium) present	Antifungal activity (Caldeira et al. 2011)
	Ferricrocin	Production (by Foc) disappeared	Sequestration of iron (Lopez-Berges et al. 2012)
	Fusaric acid	Production (by Foc) present	Antibacterial activity, sequestration of iron, quorum sensing inhibitor (Ruiz et al. 2015; Tung et al. 2017)
Foc/*Trichoderma harzianum*	Beauvericin	Production (by Foc) disappeared	Potentiator of antifungal activities of other antibiotic compounds (Wang and Xu 2012)
	Ferricrocin	Production (by Foc and *Trichoderma*) present	Sequestration of iron (Lopez-Berges et al. 2012)
	Fusaric acid	Production (by Foc) present	Antifungal activity, sequestration of iron (Ruiz et al. 2015; Son et al. 2008)
	Peptaibols (cf. Table 3)	Production (by *Trichoderma*) present	Antifungal/fungicidal activity (Benitez et al. 2004; Shi et al. 2012)

with both bacteria as well as the productions of siderophores pyoverdine E/D by *P. aeruginosa* and bacillibactin by *B. amyloliquefaciens* (Table 4) and these compounds could thus be involved in mutual competitions. The effect of the iron sequestration in the antagonistic interaction of *T. harzianum* and Foc was difficult to evaluate as the siderophore ferricrocin was produced by both fungi and, in addition, Foc maintained the production of fusaric acid (Tables 2 and 4).

The other level of the antagonist interactions included the production of specific compounds with antifungal and antibacterial activities. Here, a clear difference between the two bacteria was observed. *B. amyloliquefaciens* was able to maintain the production of bacillomycins and fengycin A, compounds shown to exhibit antifungal activities, in the presence of Foc and, at the same time, blocked the production of beauvericin by Foc, a compound having an antibacterial effect. It suggested that this bacterium was effective in the mycotoxin suppression similar to *Trichoderma* species (Blaszszyk et al. 2017). In contrast, in the case of *P. aeruginosa*, the production of bacterial rhamnolipids having an antifungal effect was blocked in the presence of Foc and the synthesis of beauvericin by Foc was maintained (Table 2). Such inhibition of rhamnolipid production in *P. aeruginosa* was reported to be mediated by microbial quorum quenching compounds (Rajesh and Rai 2016). The difference between the two bacteria may explain a higher inhibition of Foc growth by *B. amyloliquefaciens* (Fig. 1). In the co-culture with Foc, *T. harzianum* maintained the production of peptaibols exhibiting an antifungal and fungicidal activity and blocked the production of beauvericin by Foc, which resulted in a strong inhibition of Foc growth (Table 2, Fig. 1) The production of fusaric acid by Foc was present in all co-cultures with both the bacteria and *T. harzianum* suggesting that this compound with the antibacterial and antifungal activities (Son et al. 2008) was involved in the antagonistic interactions observed.

No new compounds were detected in the monocultures of the microorganisms used (compared to studies in the literature; Pathma et al. 2011); similarly, any new product was not found in the dual cultures studied. However, by comparing the antagonistic interactions of Foc with various prokaryotic and eukaryotic microorganisms, the study enabled us to observe a general pattern of response of Foc at the metabolomic level to the exposure to antagonists.

Conclusions

Fungal pathogen *F. oxysporum* f. sp. *conglutinans* race 2 (Foc) was confronted with three biological control agents of different efficiency. The observed inhibitory effect on Foc growth was in the order *T. harzianum* > *B. amyloliquefaciens* > *P. aeruginosa*. Two types of biocontrol effects were observed in bacterial and fungal antagonisms, a competition for iron mediated by siderophores and direct antibiotic effect(s) mediated by various antagonist metabolites. The efficient biocontrol activities of *T. harzianum* and *B. amyloliquefaciens* resulted from the membrane-attacking effects of peptaibols in the case of *T. harzianum* and the antifungal activity of the combination of bacillomycins and fengycin in the case of *B. amyloliquefaciens*. Simultaneously, both antagonists were also able to suppress the production of antibacterial antibiotic beauvericin by Foc. The results demonstrate that Fusarium wilt agent can be efficiently suppressed by biocontrol organisms offering thus a bioalternative to the treatment of crops by chemical pesticides.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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