doi: 10.17221/126/2016-PPS

Trichoderma atroviride Enhances Phenolic Synthesis and Cucumber Protection against *Rhizoctonia solani*

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Abstract

Nawrocka J., Szczech M., Małolepsza U. (2018): *Trichoderma atroviride* enhances phenolic synthesis and cucumber protection against *Rhizoctonia solani*. Plant Protect. Sci., 54: 17–23.

The treatment of cucumber plants with *Trichoderma atroviride* TRS25 (TRS25) provided protection against infection by *Rhizoctonia solani*. In plants inoculated with the pathogen, nontreated with *Trichoderma*, disease symptoms were observed on the roots, shoots, and leaves while in plants treated with TRS25 the spread of the disease was limited. The induction of systemic defence response in cucumber against *R. solani* infection seemed to be strongly related to the enhanced synthesis of phenolic compounds in plants. HPLC analysis indicated remarkable increases in the concentrations of 23 phenolics belonging to hydroxybenzoic acids, cinnamic acids, catechins, flavonols, flavons, and flavanons in the plants without systemic disease symptoms. We suggest that the accumulation of phenolic acids, flavonoids and *de novo* synthesis of catechins may strongly contribute to cucumber protection against *R. solani*.

Keywords: *Trichoderma* spp.; *Rhizoctonia solani*; biocontrol; defence response

Trichoderma spp. include opportunistic fungi limiting the deleterious influence of phytopathogens naturally present in soil and rhizosphere. They may either directly control pathogens or induce substantial changes in metabolism enhancing plant resistance (Hermosa *et al.* 2012; López-Bucio *et al.* 2015). Induction of the plant defence mechanisms by selected *Trichoderma* spp. is thought to be a promising plant protection strategy (Rao *et al.* 2015). *T. atroviride* is suggested to be a potential biocontrol agent against *Rhizoctonia solani* Kühn, which is one of the most destructive plant pathogens for greenhouse and field-grown cucumbers in which it causes root and shoot rot or foliar blight (Anees *et al.* 2010; Taheri & Tarighi 2011; Asad *et al.* 2014).

It is generally assumed that *Trichoderma* spp. may trigger in plants the extensive transcriptional reprogramming of genes involved in the production of phenolic compounds. Phenolics are widely distributed in cucumber, some of them occur constitutively, whereas others are synthesised in response to a pathogen attack where their appearance is considered as part of active defence (López-Bucio *et al.* 2015; Rao *et al.* 2015). Basically, their beneficial role results from antioxidant and antimicrobial properties as well as their ability to enhance plant structural barriers preventing the spread of infection (Treutter 2006; Mandal *et al.* 2010; Ghassempour 2011). There is a lack of knowledge concerning quantitative and qualitative characteristics of phenolics in plants treated with *Trichoderma* as well as their role in plant protection against *R. solani* (Mandal *et al.* 2010).

The main objective of this study was to determine the form of phenolic accumulation in *Trichoderma*

Partly supported by Priority 1.3.1, Project Polish *Trichoderma* strains in plant protection and organic waste management and co-financed by the European Union through the European Regional Development Fund within the Innovative Operational Program, 2007–2013, Project No. UDA-POIG.01.03.01-00-129/09-00.

doi: 10.17221/126/2016-PPS

TRS25-treated plants and the role of individual phenolic compounds in the limitation of *R. solani* spread.

MATERIAL AND METHODS

Microorganisms, plant material and experimental conditions. The studies were conducted with the Polish isolate of *Trichoderma atroviride* TRS25 obtained from a growth medium for fungal production and identified using an interactive key for strain identification (http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm), supported by identification keys provided by Gams and Bissett (1998) and Samuels *et al.* (2002) and molecular identification (NCBI GenBank, accession numbers: KJ786731, KJ786812) (Skoneczny *et al.* 2015). *R. solani* Kühn MUCL 47938 strain used in the experiment is a well characterised, standard phytopathogen of cucumber plants (Pannecoucque *et al.* 2008). Before its application, TRS25 isolate was determined as moderately neutral for *R. solani* growth without strong antagonistic or mycoparasitic properties (Szczech *et al.* 2014).

Cucumber (*Cucumis sativus* L.) plants cv. Iwa susceptible to *R. solani* were used in the study. The plants were grown in the medium consisting of podsolic soil and vermiculite 1 : 1 (v/v) in the growth chamber (MLR-351 H model with 15 fluorescent lamps of FL 40SSW/37 type; Sanyo, Japan), under 14 h photoperiod with a 2000 lux light intensity at 25/20°C, day/night temperature cycle and 80% relative humidity. They were watered daily with tap water. During the cultivation under controlled conditions the plants were grown with spores of TRS25 isolate at the concentration of 10^6 CFU/g of the medium. The medium without *Trichoderma* was used to grow the control plants. Three weeks after sowing half of the control and TRS25 treated plants were challenged with *R. solani* (Rs). The soil near their stem base was inoculated with 5 ml of the *R. solani* mycelial homogenate according to the method of this pathogen application presented by Pannecoucque *et al.* (2008). All plants were then maintained in the growth chamber. Four experimental groups of plants were tested:

(1) control plants – TRS25 non-treated, and non-challenged with *R. solani*
(2) Rs plants – TRS25 non-treated, and challenged with *R. solani*
(3) TRS25 plants – TRS25 treated, non-challenged with *R. solani*
(4) TRS25+Rs plants – TRS25 treated, challenged with *R. solani*

The disease symptoms were observed on the roots, shoots and leaves of five-weeks-old Rs and TRS25+Rs plants.

Biochemical analysis. For HPLC analysis phenolics were extracted according to the modified protocol of Molina *et al.* (2002). Frozen cucumber leaves were extracted three times in 80, 90, and then 100% (1 : 10; v/v) MeOH. After centrifugation (25 000 *g*, 15 min) all the supernatants were combined and evaporated to dryness under vacuum at 65°C. The residue was redissolved in water at 80°C and centrifuged (20 000 *g*, 15 min). Portions of the supernatants for free phenolic extraction were pipetted into Eppendorf tubes. To release phenolics from conjugates the aqueous phase of another part of the supernatants was acidified with HCl to pH 1 and boiled at 80°C for 1.5 h, then centrifuged (20 000 *g*, 15 min). An HPLC system (DIONEX, Sunnyvale, USA) was used for analysis. Separation of phenolics took place over an RP column (aQ Hypersil GOLD, 250 mm × 4.6 mm, 5 µm) joined with a guard column (GOLD aQ Drop-In guards, 10 mm × 4 mm, 5 µm) at 40°C, by use of a binary solvent system consisting of (A) water and (B) methanol with 0.5% formic acid (FA) with a flow rate of 1.5 ml/minute. The elution profile was as follows: 0–2 min, 40% B; 2–10 min, 40–60% B; 10–12 min, 60% B; 12–13 min, 60–40% B; 13–15 min, 40% B. Chromatograms were obtained by fluorescence monitored from 210 nm to 420 nm. In order to identify peaks in the chromatograms, the retention times, co-injections and spectra of the studied samples were compared with pure phenolic reference standards of phenolic acids, flavonoids and their derivatives according to previous reports (Keski-Saari & Julkunen-Tiitto 2003; Kelebek *et al*. 2009). An overview of all the characterised compounds in the cucumber leaf extracts is given in Table 2. The phenolic contents were expressed as µg of respective standards per g of FW.

The effect of the detected phenolics on the growth of *R. solani* mycelium was evaluated in *in vitro* assays. The compound concentrations were the same as those observed in TRS25+Rs plants. Petri dishes containing 15 ml of potato dextrose agar (PDA) were used. Solutions of phenolics were poured into cut-out holes. 80% methanol was used as control. Each Petri dish was inoculated in the centre with 1 cm disc of one-month-old fungus mycelium. All inoculated plates were incubated for five days at 22°C. Linear mycelial growth of pathogenic fungi was evaluated every 24 hours.

doi: 10.17221/126/2016-PPS

Statistical analysis. Samples for determination of biochemical parameters were analysed in triplicate from three independent experiments. The results are given as means ± SD. The nonparametric Kruskal-Wallis test was used for statistical evaluation where $P < 0.05$ was considered statistically significant and marked using * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$.

RESULTS

T. atroviride TRS 25 when applied into the growth medium significantly reduced *R. solani*-induced disease symptoms on the cucumber roots from average 60 ± 1% to 16 ± 2%, on the shoots from average 42 ± 4% to 11 ± 2% and on the leaves from average 28 ± 1% to 5 ± 0.2% in TRS25+Rs plants as compared to Rs plants (Table 1).

In the plants with decreased disease symptoms systemic accumulation of phenolics was observed. Significant increases in the levels of 23 phenolics in TRS25 and TRS25+Rs plants as compared to the control and Rs plants were revealed using the HPLC analysis (Table 2). In the group of hydroxybenzoic acid (hBA) derivatives, noteworthy accumulation of vanillic, syringic, resorcylic, ellagic, and salicylic acids was observed. Among these compounds, in TRS25 plants the most considerable more than fourfold increase in the concentration of resorcylic acid was detected as compared to the control (Table 2). In TRS25+Rs plants the most significant more than sixfold and twofold increases were observed in resorcylic and salicylic acid concentrations, respectively, as compared to Rs plants. Additionally to hBA derivatives, the accumulation of all detected cinnamic acid derivatives, starting from *t*-cinnamic acid through different isoforms of coumaric acid, caffeic and ferulic acids to chlorogenic and caffeoylquinic acids was observed in TRS25 and TRS25+Rs plants as compared to the control and Rs plants (Table 2). While in TRS25 plants the ninefold and fourfold increases in *p*- and *o*-coumaric acid concentrations, respectively, were most pronounced as compared to the control, in TRS25+Rs plants the significant threefold increase of coumaric acid isoform concentrations was accompanied by strongly enhanced caffeic and ferulic acid concentrations as compared to Rs plants.

Table 1. Rating of the disease caused by *R. solani*

Disease rating (%)	Control	Rs	TRS25	TRS25+Rs
Cucumber roots	nd	60 ± 1	nd	16 ± 2***
Cucumber shoots	nd	42 ± 4	nd	11 ± 2**
Cucumber leaves	nd	28 ± 1	nd	5 ± 0.2**

Values represent the means ± SD from three independent experiments with three replicates each; Rs – plant inoculated with *R. solani*; TRS25 – plant treated with *Trichoderma*; TRS25+Rs – plant treated with *Trichoderma* and inoculated with *R. solani*; nd – not detected; significant differences between Rs and TRS25+Rs plants were marked using ** for $P < 0.01$ and *** for $P < 0.001$

The mean levels of flavonoids were significantly higher in TRS25 and TRS25+Rs plants as compared to the control and Rs plants (Table 2). Among them, the most considerable sevenfold and sixfold increases in the concentration of coumarin and naringenin accompanied by a fourfold increase in luteolin and twofold increases in 3-hydroxyflavone and hesperetin were observed in TRS25 plants as compared to the control. The significant increases of these compounds, with the exception of hesperetin, were also observed in TRS25+Rs plants, and they were accompanied by an almost fivefold increase in quercetin content in comparison with Rs plants. In the group of flavonoids including the catechin subgroup, four compounds were not detected in the control and Rs plants while their presence was irrefutable in TRS25 plants. The following concentrations of three compounds, i.e. myricetin, (–)-epigallocatechin and (–)-epicatechin (1.98 ± 0.33, 0.46 ± 0.11, and 0.21 ± 0.03 g/g FW, respectively) were first detected in TRS25 plants while they were significantly higher in TRS25+Rs plants (2.91 ± 0.27, 1.82 ± 0.32, and 0.74 ± 0.02 µg/g FW, respectively) and accompanied by (+)-catechin (0.53 ± 0.02 µg/g FW) occurrence. *De novo* synthesis of the above-mentioned compounds occurred in parallel with the sixfold and threefold increases in pyrocatechol and pyrocatechin concentrations detected both in TRS25 and TRS25+Rs plants as compared to the control and Rs plants.

In order to test the ability of accumulated phenolics to inhibit the *R. solani* mycelium growth, a microbiological assay was performed. Generally, the tested phenolics effectively suppressed the pathogen growth (Table 3). The measurement of the *R. solani* mycelium growth repeated every 24 h showed different effects of the tested compounds on this parameter after 72 hours. The final measurement performed 120 h after *R. solani* passage showed that some flavonoids and catechins, especially (+)-catechin, 3-hydroxyflavone, pyrocatechol, myricetin

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Table 2. Effect of *T. atroviride* TRS25 on phenolic concentrations in cucumber leaves detected by HPLC analysis

	Retention time (min)	Phenolic content (μg/g FW)			
		control	Rs	TRS25	TRS25+Rs
Hydroxybenzoic acid derivatives					
Gallic acid	7.37	0.11 ± 0.03	0.20 ± 0.03	0.11 ± 0.01	0.24 ± 0.17
Gentisic acid	16.85	0.20 ± 0.09	0.16 ± 0.03	0.35 ± 0.10	0.37 ± 0.07
Vanillic acid	18.65	0.62 ± 0.18	0.95 ± 0.11	1.97 ± 0.35*	1.76 ± 0.23*
Syringic acid	19.32	1.34 ± 0.71	1.35 ± 0.10	2.28 ± 0.14*	3.79 ± 0.51*
Resorcylic acid	19.38	0.91 ± 0.13	0.78 ± 0.17	3.96 ± 0.98**	5.23 ± 0.97**
Ellagic acid	27.45	0.37 ± 0.10	0.59 ± 0.13	1.61 ± 0.51*	1.65 ± 0.12*
Salicylic acid	29.25	9.34 ± 1.12	5.54 ± 0.17	13.35 ± 2.3*	13.90 ± 3.1**
Cinnamic acid derivatives					
Chlorogenic acid	17.73	0.14 ± 0.04	0.26 ± 0.01	0.52 ± 0.05*	0.40 ± 0.03*
Caffeic acid	19.05	0.41 ± 0.03	1.29 ± 0.10*	0.94 ± 0.14*	2.86 ± 0.32**
Ferulic acid	19.95	5.77 ± 0.11	8.10 ± 1.01	13.56 ± 2.0*	16.56 ± 2.6**
Caffeoylquinic acid	20.04	0.59 ± 0.03	0.49 ± 0.08	1.41 ± 0.17 *	0.92 ± 0.12*
o-Coumaric acid	23.30	2.36 ± 0.13	4.51 ± 0.21	9.50 ± 1.02**	11.17 ± 1.3***
p-Coumaric acid	26.00	0.76 ± 0.05	2.53 ± 0.11*	6.77 ± 0.22***	4.54 ± 0.41**
t-Cinnamic acid	33.33	0.32 ± 0.04	0.57 ± 0.01	2.35 ± 0.13*	2.59 ± 0.87*
Flavonoids					
Coumarin	25.46	0.80 ± 0.22	2.07 ± 0.34	5.88 ± 2.3**	8.89 ± 1.82***
Rutin	27.26	0.38 ± 0.11	0.45 ± 0.66	0.70 ± 0.17	0.89 ± 0.19
Myricetin	28.95	nd	0.09 ± 0.01	1.98 ± 0.33***	2.91 ± 0.27***
Quercetin	32.92	0.15 ± 0.03	0.12 ± 0.01	0.23 ± 0.02	0.55 ± 0.04**
Naringenin	33.75	0.20 ± 0.06	0.51 ± 0.03	1.17 ± 0.87**	0.60 ± 0.34*
Luteolin	34.21	0.13 ± 0.01	0.11 ± 0.03	0.54 ± 0.08*	0.51 ± 0.06*
Hesperetin	34.50	0.72 ± 0.03	0.75 ± 0.14	1.69 ± 0.32*	0.51 ± 0.19
Kaempferol	36.56	0.16 ± 0.02	0.08 ± 0.00	0.17 ± 0.03	0.11 ± 0.00
Apigenin	37.28	0.14 ± 0.02	0.09 ± 0.00	0.12 ± 0.03	0.21 ± 0.04
3-Hydroxyflavone	48.78	11.23 ± 2.3	14.85 ± 1.8	25.62 ± 4.1**	28.03 ± 3.1***
Catechins					
(−)-Epigallocatechin	12.06	nd	nd	0.46 ± 0.11*	1.82 ± 0.32**
Pyrocatechol	13.06	0.21 ± 0.03	0.16 ± 0.01	1.17 ± 0.21**	0.91 ± 0.09**
o-Pyrocatechin	15.46	0.97 ± 0.66	2.54 ± 0.38	3.21 ± 0.99*	6.87 ± 1.13*
(+)-Catechin	15.73	nd	nd	nd	0.53 ± 0.02***
(–) Epicatechin	19.45	nd	nd	0.21 ± 0.03*	0.74 ± 0.02**

Values represent the means ± SD from three independent experiments with three replicates each; Rs – plant inoculated with *R. solani*; TRS25 – plant treated with *Trichoderma*; TRS25+Rs – plant treated with *Trichoderma* and inoculated with *R. solani*; nd – not detected; significant differences between Rs and TRS25+Rs plants were marked using * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$

and (–)-epigallocatechin were the most effective inhibitors of mycelium growth and reduced it to 51 ± 3, 54 ± 4, 56 ± 3, 63 ± 6, and 68 ± 1% of the control (100%), respectively. Comparatively, in the presence of phenolic acids, i.e. resorcylic, ferulic, *p*- or *o*-coumaric acids, the *R. solani* mycelium growth oscillated around 70 ± 2, 73 ± 1, 77 ± 2%, and 78 ± 2% of the control, respectively.

DISCUSSION

The obtained results showed that an exposure of cucumber roots to *T. atroviride* TRS25 isolate significantly reduced the severity of root, shoot, and leaf disease symptoms caused by *R. solani*. As documented by Szczech *et al.* (2014), TRS25 is characterised by the production of chitinases, glucanases and volatiles.

doi: 10.17221/126/2016-PPS

Table 3. Inhibition of the *R. solani* mycelium growth by phenolics isolated from the leaves of TRS25+Rs plants

Phenolic compound (µg/g FW)	Growth of *R. solani* mycelium (%)			
	48 h	72 h	96 h	120 h
Control	21 ± 1	35 ± 2	72 ± 3	100 ± 0
m-Coumaric acid	11 ± 3	17 ± 2	30 ± 4	78 ± 2
p-Coumaric acid	21 ± 1	27 ± 3	62 ± 2	77 ± 2
Caffeic acid	15 ± 2	28 ± 4	68 ± 3	88 ± 3
Ferulic acid	20 ± 1	22 ± 1	59 ± 2	73 ± 1
Resorcylic acid	18 ± 4	29 ± 2	48 ± 3	70 ± 2
Salicylic acid	15 ± 3	25 ± 4	51 ± 3	90 ± 6
3-Hydroxyflavone	17 ± 0	24 ± 2	50 ± 1	54 ± 4
Pyrocatechol	16 ± 1	17 ± 3	27 ± 1	56 ± 3
(–)-Epigallocatechin	20 ± 2	28 ± 2	55 ± 1	68 ± 1
(+)-Catechin	17 ± 2	20 ± 1	44 ± 7	51 ± 3
(–)-Epicatechin	15 ± 3	27 ± 2	55 ± 4	80 ± 4
Myricetin	13 ± 2	18 ± 2	43 ± 3	63 ± 6
Quercetin	14 ± 1	27 ± 1	47 ± 4	89 ± 7

Values represent the means ± SD from three independent experiments with three replicates; compound concentrations were the same as those observed in the TRS25 treated, challenged with *R. solani* (TRS25+Rs) plants present in Table 2

Although those compounds had a moderate influence on the *R. solani* mycelium growth, they could act as elicitors which activate the expression of genes involved in the plant systemic resistance. In the tested plants the induction of systemic defence response to the *R. solani*-induced disease seemed to be strongly related to biocontrol activity of the TRS25 isolate. Special attention was paid to phenolic metabolism as its role in plant defence against pathogenic fungi was emphasised previously but not extensively studied for the *Trichoderma-R. solani* interaction (Rao *et al.* 2015; López-Bucio *et al.* 2015).

Considerable increases in the concentrations of phenolics being hBA and cinnamic acid derivatives, flavonoids, and catechins were observed in TRS25 and TRS25+Rs plants. Although TRS25 and *R. solani*, being in the soil, directly influenced only the roots of plants, the aboveground parts in which the phenolic accumulation was observed were protected against the disease. According to Mandal *et al.* (2010) in such a case cucumber protection may be regulated by a network of signal transduction pathways in which phenolic acids, in a conjugated form with a sugar residue or as esters, are key signalling molecules. In the present study, accumulated phenolic acids with resorcylic and salicylic acid prevailing among hBA derivatives as well as coumaric, caffeic, and ferulic acid among cinnamic acid derivatives might be considered as resistance signalling molecules as it was also suggested by Contreras-Cornejo *et al.* (2011) for the protection of *Arabidopsis thaliana* against *B. cinerea* by *T. virens* and *T. atroviride*. In this case, TRS25 seemed to regulate the phenylpropanoid biosynthesis in the plants where coumaric and ferulic acids are the important precursors for toxic quinone, tannin, lignin, and cinnamyl alcohol biosynthesis (Mandal *et al.* 2010; Vogt 2010). These compounds might be used to inhibit directly the pathogen growth and to reinforce the cell wall, supporting mechanical plant protection (Treutter 2006; Castellano *et al.* 2012).

In addition to mechanical protection against the disease spread, flavonoids accumulated in TRS25-treated plants may be simultaneously involved in triggering a systemic biochemical response in cucumber. Since it is known that these compounds are very important in plant resistance to pathogenic bacteria and fungi (Treutter 2006; Mandal *et al.* 2010), we can assume that the intensively accumulated coumarin, naringenin, luteolin, 3-hydroxyflavone, and quercetin play an important role in plant protection against *R. solani* in the present experiment. Flavonoids inhibit a number of root pathogens, especially the fungal ones. Studies on barley mutants showed that proanthocyanidins or dihydroquercetin, even in small amounts, were involved in the protection of plants against *Fusarium* sp. (Mierziak *et al.* 2014). Anti-pathogenic properties of these compounds are non-specific and result, in part, from their antioxidative properties (Pourcel et al. 2006; Treutter 2006). They may quench reactive oxygen species, which are generated both by the pathogen and the plant as a result of infection. Moreover, flavonoids can contribute to the tightening of plant structures and tissues by modulating auxin (IAA) activity; this can lead to differentiation of tissues, promotion of callus and tylose formation and closure of the vascular system to prevent the pathogen infection (Mierziak *et al.* 2014).

Additionally to the enhancement of the content of constitutive flavonoids, inoculation of plants with microorganisms may result in *de novo* synthesis of phenolics during the progress of plant-microorganism interaction (Mandal *et al*. 2010; Vogt 2010). In the present study, the analysis revealed four flavonoids, i.e. myricetin, (–)-epigallocatechin, (+)-catechin, and (–)-epicatechin, which seem to be synthesized additionally in TRS25+Rs plants but not in the control and Rs plants. Catechin and epicatechin belong to

doi: 10.17221/126/2016-PPS

the class of flavan-3-ols which are predominantly synthesized as (+)-catechin and (–)-epicatechin enantiomers and in this form have different physiological and biological effects. Together with pyrocatechol, they may show antioxidant and antifungal properties as well as be monomer units of condensed tannin (Ghassempour *et al.* 2011; Girardi *et al.* 2014). As the most pronounced synthesis of different forms of catechins was observed in TRS25+Rs plants, their important role in preventing the spread of *R. solani* infection may be considered.

Generally, many phenolics can act directly against pathogens (Treutter 2006; Mandal *et al.* 2010). Yamamoto *et al.* (2000) reported that among different phenolics catechin, especially in the form of (+)catechin, produced in strawberry leaves was the main infection inhibiting factor. Moreover, catechin and epicatechin showed antifungal activity against *Alternaria* sp. and *Fusarium* sp. as described by Girardi *et al.* (2014). In the present study, some flavonoids and catechins, especially (+)-catechin and (–)-epigallocatechin, synthesised *de novo* and 3-hydroxyflavone, pyrocatechol and myricetin significantly accumulated in TRS25+Rs plants were shown to strongly supress the *R. solani* mycelium growth. The above-mentioned compounds were more toxic to the pathogen than the tested hBA and cinnamic acids, therefore their significant role in direct plant protection against *R. solani* may be suggested. According to Wu *et al.* (2013) and Mierziak *et al.* (2014) direct effects of flavonoids may be associated with the inhibition of spore germination and of mycelium hyphal elongation by disruption of microbial membranes and changing their fluidity. The B ring of flavonoids can intercalate or form hydrogen bonds with the stacks of nucleic acid bases, which further leads to the inhibition of DNA and RNA synthesis in microorganisms. Moreover, they may inhibit the pathogen's enzymes digesting the plant cell wall. The inhibitory effect of the selected compounds observed in the present study confirms the results presented by Treutter (2006), who revealed that catechins and flavones inhibited fungal pectinase and cellulase activity as well as inhibited the growth of different fungi *in vitro*. The role of accumulated phenolics as molecules which regulate genes responsible for establishing the molecular dialog of plants with biological control agents including *Trichoderma* spp. may also be considered. In this case, hBA, *p*-coumaric acid or quercetin accumulated in TRS25 and TRS25+Rs plants might have a similar resistance stimulatory effect on plant treatment with TRS25 strain as it was described by Mandal *et al.* (2010) in clover and sorghum roots treated with arbuscular mycorrhizal fungi.

On the basis of the obtained data, it can be assumed that *T. atroviride* TRS25 plays an important role in the protection of cucumber plants against *R. solani*. The induction of systemic defence response in cucumber may be considered as part of the biocontrol activity of the isolate. The accumulation of cinnamic and hydroxybenzoic acid derivatives and flavonoids from different groups with *de novo* synthesis of catechins may enhance protective barriers of plants at biochemical and molecular levels. TRS25 stimulates the accumulation of phenolic acids, especially resorcylic and salicylic ones, which may participate in cucumber defence response to the *R. solani*-induced disease. Moreover, some phenolics, especially (+)-catechin, (–)-epigallocatechin and myricetin, synthesised *de novo* as well as 3-hydroxyflavone and pyrocatechol constantly produced in cucumber plants may strongly suppress the growth of the pathogen. The present results not only expand the number of known and predicted secondary metabolites from the phenolic group accumulated in the cucumber plants treated with *Trichoderma* but also show the critical role of these compounds with antimicrobial activity in the indirect and direct protection against fungal pathogens. TRS25 seems to be a potential biocontrol agent for disease management in crop plants.

Acknowledgements. The authors are grateful to Ms M. Fronczak for her linguistic correction of the manuscript and Ms. Wielanek for help in HPLC analyses.

References

Anees M., Edel-Hermann V., Steinberg C. (2010): Build up of patches caused by *Rhizoctonia solani*. Soil Biology and Biochemistry, 42: 1661–1672.

Asad S.A., Ali N., Hameed A., Khan S.A., Ahmad R., Bilal M., Shahzad M., Tabassum A. (2014): Biocontrol efficacy of different isolates of *Trichoderma* against soil borne pathogen *Rhizoctonia solani*. Polish Journal of Microbiology, 63 (1): 95–103.

Castellano G., Tena J., Torres F. (2012): Classification of phenolic compounds by chemical structural indicators and its relation to antioxidant properties of *Posidonia oceanica* (L.) Delile. MATCH Communications in Mathematical and in Computer Chemisty, 67: 231–250.

Contreras-Cornejo H.A., Macías-Rodríguez L., Beltrán-Peña E., Herrera-Estrella A., López-Bucio J. (2011).

doi: 10.17221/126/2016-PPS

Trichoderma-induced plant immunity likely involves both hormonal- and camalexin-dependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungus *Botrytis cinerea*. Plant Signaling & Behavior, 6: 1554–1563.

Gams W., Bissett J. (1998): Morphology and identification of *Trichoderma*. In: Kubicek C.P., Harman G.E. (eds): *Trichoderma* and *Gliocladium*. Basic Biology, Taxonomy and Genetics. London, Taylor & Francis: 3–34.

Ghassempour A., Mollayi S., Farzaneh M., Sharifi-Tehrani A., Aboul-Enein H.Y. (2011): Variation of catechin, epicatechin and their enantiomers concentrations before and after wheat cultivar-*Puccinia triticina* infection. Food Chemistry, 125: 1287–1290.

Girardi F.A., Tonial F., Chini S.O., Sobottka A.M., Schefer-Basso S.M., Bertol C.D. (2014): Phytochemical profile and antimicrobial properties of *Lotus* spp. (Fabaceae). Anais da Academia Brasileira de Ciências, 86: 1295–1302.

Hermosa R., Viterbo A., Chet I., Monte E. (2012): Plant-beneficial effects of *Trichoderma* and its genes. Microbiology, 158: 17–25.

Kelebek H., Selli S., Canbas A., Cabaroglu T. (2009): HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. Microchemical Journal, 91: 187–192.

Keski-Saari S., Julkunen-Tiitto R. (2003): Resource allocation in different parts of juvenile mountain birch plants: effect of nitrogen supply on seedling phenolics and growth. Physiological Plantarum, 118: 114–126.

López-Bucio J., Pelagio-Flores R., Herrera-Estrella A. (2015): *Trichoderma* as biostimulant: exploiting the multilevel properties of a plant beneficial fungus. Scientia Horticulturae, 196: 109–123.

Mandal S.M., Chakraborty D., Dey S. (2010): Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signaling & Behavior, 5: 359–368.

Mierziak J., Kostyn K., Kulma A. (2014): Flavonoids as important molecules of plant interactions with the environment. Molecules, 19: 16240–16265.

Molina A., Bueno P., Marín M.C., Rodríguez-Rosales M.P., Belver A., Venema K., Donaire J.P. (2002): Involvement of endogenous salicylic acid content, lipoxygenase and antioxidant enzyme activities in the response of tomato cell suspension cultures to NaCl. New Phytologist, 156: 409–415.

Pannecoucque J., Van Beneden S., Höfte M. (2008): Characterization and pathogenicity of *Rhizoctonia* isolates associated with cauliflower in Belgium. Plant Pathology, 57: 737–746.

Pourcel L., Routaboul J.-M, Cheynier V., Lepiniec L., Debeaujon I. (2006): Flavonoid oxidation in plants: from biochemical properties to physiological functions. Trends in Plant Science, 12: 29–36.

Rao G.S., Reddy N.N.R., Surekha C. (2015): Induction of plant systemic resistance in Legumes *Cajanus cajan*, *Vigna radiata*, *Vigna mungo* against plant pathogens *Fusarium oxysporum* and *Alternaria alternata* – a *Trichoderma viride* mediated reprogramming of plant defense mechanism. International Journal of Science and Research, 6: 4270–4280.

Samuels G.J., Dodd S.L., Gams W., Castelbury L.A., Petrini O. (2002): *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. Mycologia, 94: 146–170.

Skoneczny D., Oskiera M., Szczech M., Bartoszewski G. (2015): Genetic diversity of *Trichoderma atroviride* strains collected in Poland and identification of loci useful in detection of within-species diversity. Folia Microbiologica, 60: 297–307.

Szczech M., Witkowska D., Piegza M., Kancelista A., Małolepsza U., Gajewska E., Kowalska B. (2014): Selection system for beneficial microorganisms following *Trichoderma* example. In: 11th Conference of the European Foundation for Plant Pathology Healthy Plants – Healthy People. Sep 8–13, 2014, Kraków, Poland: 301.

Taheri P., Tarighi S. (2011): A survey on basal resistance and riboflavin-induced defense responses of sugar beet against *Rhizoctonia solani*. Journal of Plant Physiology, 168: 1114–1122.

Treutter D. (2006): Significance of flavonoids in plant resistance: a review. Environmental Chemistry Letters, 4: 147–157.

Wu T., Zang X., He M., Pan S., Xu X. (2013): Structure-activity relationship of flavonoids on their anti-*Escherichia coli* activity and inhibition of DNA gyrase. Journal of Agricultural and Food Chemistry, 61: 8185–8190.

Vogt T. (2010): Phenylpropanoid biosynthesis. Molecular Plant, 3: 2–20.

Yamamoto M., Nakatsuka S., Otani H., Kohmoto K., Nishimura S. (2000): (+)-Catechin acts as an infection-inhibiting factor in strawberry leaf. Biochemistry and Cell Biology, 90: 595–600.

Received: 2016–08–31
Accepted after corrections: 2017–02–23
Published online: 2017–04–13