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The secretion pattern of root exudates in tomato plants inoculated with *Botrytis cinerea* results in higher populations of *Trichoderma asperellum* strain T34 in the rhizosphere

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Running Head: Leaf to root communication

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Key words: induced systemic resistance, gluconic acid, biological control agent, organic acids.

Abstract

Root exudates secreted from plants can modify rhizosphere microbiota by enhancing or inhibiting the growth of Biological control agents (BCAs) and/or pathogens. Similarly, microorganisms can modify the secretion of plant root exudates. The aim of this study was to analyse the effect of a *Botrytis cinerea* leaf infection on the secretion of tomato root exudates and on the populations of the BCA *Trichoderma asperellum* strain T34 (T34).

This study found that the secretion pattern of root exudates in tomato plants was influenced by *B. cinerea* infection in plant leaves. An increase in the levels of gluconic acid was observed, while levels of sucrose and inositol decreased. A decrease in the severity of *B. cinerea* by the induction of systemic resistance triggered by T34 was also observed. Tomato plants infected with *B. cinerea* maintained the populations of T34 in the roots, while populations of T34 decreased in plants not inoculated with the pathogen. Samples exposed to media containing gluconic acid (as the only carbon source or at the same concentration found in roots exudates) saw an increase in the *in vitro* growth of T34 compared to media without gluconic acid. In conclusion, a change in the secretion pattern of root exudates caused by *B. cinerea* together with the enhanced growth of T34 in the presence of gluconic acid, indicates the existence of leaf to root communication. The result of which enhances the populations of T34 and in turn induces disease resistance and a consequential reduction in disease severity.

1. Introduction

The use of biological control measures has been promoted in recent years due to an increased awareness of the noxious effect on the environment of chemical pesticides and the emergence of resistance to these pesticides. The current European Directive (2009/128/EC) addresses the sustainable use of pesticides which includes promoting the use of integrated pest management and the use of alternative approaches to chemical pesticides such as biological control agents (BCAs).

Botrytis cinerea Persoon: Fries, teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel is a necrotrophic plant pathogen which causes a grey mould disease. *B. cinerea* was ranked as the second most important pathogenic plant fungus due to its economic impact and the capacity to infect more than 200 plant species (Dean *et al.*, 2012). Normally the disease is controlled with fungicides (specific and broad spectrum), however, *B. cinerea* has developed resistance to most of them (Leroch *et al.*, 2011).

Mechanisms to control foliar diseases used by bacterial and fungal BCAs are: i) direct action of BCA over the pathogen; or ii) indirect action of BCA through the plant by the induction of systemic resistance and/or the improvement of plant nutrition and growth. The genus *Trichoderma* spp. is one of the most isolated soil fungi and has been used as a biopesticide, a biofertilizer and in soil amendments (Vinale *et al.*, 2008). According to Verma *et al.* (2007), the typical biocontrol actions of *Trichoderma* spp. are mycoparasitism, spatial and nutrient competition and antibiosis. Some strains of *Trichoderma* spp. can induce systemic resistance (Pieterse *et al.*, 2014) and improve plant nutrition and growth (Li *et al.*, 2015). *Trichoderma asperellum* strain T34 (T34) induced systemic resistance against different foliar pathogens in cucumber plants

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against *Pseudomonas syringae* pv. *lachrymans* (Segarra *et al.*, 2007); in *Arabidopsis* against *Pseudomonas syringae* pv. *tomato*, *Hyaloperonospora parasitica* and *Plectosphaerella cucumerina* (Segarra *et al.*, 2009); and in the tomato plant, it induced systemic resistance against *B. cinerea* as well as increasing plant growth and nutrient uptake (Fernández *et al.*, 2014).

In this study, given their global economic significance, tomato (*Lycopersicon esculentum* [Miller, 1768], *Solanum lycopersicum* [Linné, 1753]) was used; this plant is susceptible to *B. cinerea* and is inducible by T34, which triggers the systemic resistance response against attack by *B. cinerea* (Fernández *et al.*, 2014).

Plants can maintain positive (symbiotic and protective relationships) and negative interactions (pathogenic associations) with other plants, microbes and invertebrates through selective root exudation of specific compounds (Bais *et al.*, 2006; Haichar *et al.*, 2014). According to Bertin *et al.* (2003), root exudates mainly contain water, ions, amino acids, organic acids, sugars, enzymes and phenolic compounds. Nevertheless, the quality and quantity of root exudation depend on characteristics of the plant itself (root growth, development stage of plants, plant species and genotypes within species) and external biotic and abiotic factors (Badri & Vivanco, 2009).

The presence of pathogens and BCAs – in isolation or together – modifies the way in which root exudates are secreted. This was observed in tomato root exudates in the presence and absence of the pathogen *Fusarium oxysporum* f. sp. *radicis-licopersici* (Forl) and the BCA *Pseudomonas fluorescens* WCS365 (Kamilova *et al.*, 2006b). Similarly, root exudates of tomato plants are altered differently by pathogenic and non-pathogenic *F. oxysporum* strains, and consequently, the propagation of every strain in

the rhizosphere is affected differently (Steinkellner *et al.*, 2008). Furthermore, exudate profiles of Arabidopsis plants change in the presence of the wild-type *Pseudomonas putida* KT2440 and the mutant PP2561, which is negative in the induction of resistance against the foliar pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (Pst D3000) (Matilla *et al.*, 2010). Interestingly, Arabidopsis plants increase secretions of L-malic acid induced by the foliar pathogen Pst D3000 that promote binding and biofilm formation of the beneficial rhizobacterium *Bacillus subtilis* FB17 on roots (Rudrappa *et al.*, 2008). This demonstrates the importance of leaf to root communication, the versatile interactions between microorganisms and plants through root exudates, and the crucial role they could play in plant defence.

Moreover, there is evidence that tomato plants may secrete certain compounds through exudation to attract beneficial microorganisms. Organic acids from tomato root exudates influence root colonisation, chemotaxis and swarming motility by the BCA *Bacillus amyloliquefaciens* T-5 (Tan *et al.*, 2013). The antifungal activity against Forl of the plant growth promoting rhizobacteria *Pseudomonas chlororaphis* SPB1217 and *P. fluorescens* SPB2137 depend on the sugar and organic acid composition of tomato root exudates (Kravchenko *et al.*, 2003).

There is little information available about the interaction between *Trichoderma* spp. and root exudates. According to Zhang *et al.* (2013), *Trichoderma harzianum* T-E5 modified cucumber root exudates and these exudates reduced the germination of the soil pathogen *Fusarium oxysporum* f. sp. *cucumerinum*. Moreover, root exudates of Bengal gram can promote the growth of some isolates of *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma roseum* (Jash & Pan, 2007). Information is scarce on the role of root exudates in plant defence against foliar pathogens, how plants encourage beneficial

microorganisms to help them in response to pathogen attack, and the responses that can be triggered in beneficial microorganisms.

The objective of this study was to analyse the effect of *B. cinerea* leaf infection on the secretion pattern of tomato root exudates and its connection with the populations of the BCA *T. asperellum* strain T34 and disease reduction.

2. Materials and methods

2.1. Study in sterile conditions

To obtain root exudates in sterile conditions, the setup of the experiment was adapted from Kamilova *et al.* (2006a). Tomato (*Solanum lycopersicum* L.) cv. Roma seeds were surface sterilised by immersion in 5% HClO for 3 min and subsequently washed with sterile distilled water (H₂O_d). Seeds were placed in sterile 500 mL glass jars under sterile conditions containing a 2 cm thick layer of sand on the bottom. The sand was saturated with 50 mL nutrient solution (0.5 g L⁻¹ Peter's foliar feed 27-15-12 from Scotts, 0.22 g L⁻¹ CaCl₂ and 0.25 g L⁻¹ MgSO₄·7 H₂O). Jars were kept at 24 °C (day) and 20 °C (night) with 16 h light. Two weeks later, in half of the jars, the seedlings were inoculated with a *B. cinerea* and the inoculum was adjusted to 10⁵ conidia mL suspension prepared as described in Fernández *et al.* (2014) with the exception that the inoculation buffer did not contain glucose. Two weeks after the inoculation, the nutrient solution was removed from the sand and lyophilised.

Samples for root exudates were processed and analysed by gas chromatography/mass spectrometry (GC/MS) as described by Matthew *et al.* (2009). Ribitol ($100 \mu\text{g g}^{-1}$) was used as the internal standard. Retention index was calculated by the use of an alkane standard mix containing $100 \mu\text{g g}^{-1}$ of C12, C15, C19, C22, C28, C32, and C36.

For GC/MS Instrument Settings, the samples were randomised, and a splitless injection was used to move $1 \mu\text{L}$ of derivatized sample into a Shimadzu QP2010 GC-MS Gas Chromatograph-Mass Spectrometer. Helium was used as the carrier gas at a constant flow of 1 mL min^{-1} . The inlet temperature was set at $280 \text{ }^\circ\text{C}$. The oven temperature was initially set at $70 \text{ }^\circ\text{C}$ for 1 min, ramped at $1 \text{ }^\circ\text{C min}^{-1}$ until $76 \text{ }^\circ\text{C}$, then ramped at $6 \text{ }^\circ\text{C min}^{-1}$ until $325 \text{ }^\circ\text{C}$, with a final hold of 8 min. A Tracer capillary column (TRB-5 ms, 30 m _ 0.25 mm, 0.25_m) was used. The mass selective detector transfer line heater was kept at $300 \text{ }^\circ\text{C}$ and MS and source temperature at $200 \text{ }^\circ\text{C}$. Mass detection range was set from 40 to 900 atomic mass units. For GC/MS Metabolite Peak Identification and Quantification, the metabolites were identified by retention index and spectral comparison to pre-run standards or by searching the NIST library. Normalisation was performed to the internal standard ribitol. Due to the high number of unidentified peaks an alternative approach was used to analyse the data output from the GC/MS experiments: peaks were aligned based on retention index, their areas were normalised according to the internal standard and a principal component analysis (PCA) was performed.

2.2. Study of *T. asperellum* strain T34 growth rate

To assess the capacity of gluconic acid to promote T34 growth, the growth media used were: cornmeal dextrose agar (CMD, Difco Cornmeal agar and 20 g L⁻¹ dextrose) and three modified versions of Synthetic Nutrient-poor Agar (SNA) described by Nirenberg (1981) with different sugar composition (SNA1 [0.2 g L⁻¹ sucrose and 0.2015 g L⁻¹ dextrose, pH = 5.7, SNA2 [0.2 g L⁻¹ sucrose, 0.2 g L⁻¹ dextrose and 0.0015 g L⁻¹ gluconic, pH = 5.7] and SNA3 [0.4015 g L⁻¹ gluconic, pH = 4.1]). Plastic petri dishes with a diameter of 9 cm and 20 mL of fresh medium from plate were used.

First, T34 stored in silica gel crystals at 4 °C was cultivated in CMD for 3 days at 25 °C under dark conditions. Following this, plugs of 5 mm of diameter were obtained from the actively growing edge of the colony and were placed mycelium-side-down at approximately 1.5 cm from the edge of the plate in SNA 1, SNA 2 and SNA 3. Plates were incubated for 5 days at 30 °C under dark conditions. A total of 9 plates per growth media were used and the experiment was repeated two times.

The growth rates were evaluated every 24 h from day 2 to 4 by measuring the colony radius from the middle of the inoculum plug. A visual inspection of the colony growth was also made.

2.3. Study in growth chamber

To evaluate T34 as an inducer of plant disease resistance against *B. cinerea*, tomato seeds cv. Roma were pre-germinated with sterile H₂O_d and paper for 4 days at 25 ± 3 °C and dark conditions. Germinated seeds were sown for 14 days in 200 mL pots in perlite, (Europerlite) and perlite enriched with T34 (P+T34). To prepare P+T34, perlite was inoculated with T34 to achieve an average concentration of 3.85 ± 0.53 × 10⁵ CFU cm⁻³ growth media at the point of inoculation with the pathogen (day 0). Pots were placed in a growth chamber (25 ± 2 °C, 16 h of light at 100–120 μmol m⁻² s⁻¹ photosynthetic photon flux density and 60-80% relative humidity). Plants were irrigated on the media as required (17.5 solution mL⁻¹ medium day⁻¹ in the pot) with the following nutrient solution: 0.2 g L⁻¹ Peter's Foliar Feed 27-15-12 (Scotts), 0.09 g L⁻¹ CaCl₂ and 0.05 g L⁻¹ MgSO₄ 7 H₂O.

On day 14 plants were placed in mini-tunnels (inside the growth chamber) to establish optimal conditions for *B. cinerea* disease (Fernández *et al.*, 2014). The number of plants in each of the two growth media was 16. There were 8 (control) plants that had not been inoculated with the pathogen and 8 plants with pathogen-inoculated leaves. The control plants were placed in separate mini-tunnels to the inoculated plants, each of which was grown in a different mini-tunnel. The experiment was repeated two times. Two expanded leaves from each plant were sprayed (low pressure plastic hand sprayer) with approximately 550 μL per leaf of inoculation buffer (De Meyer *et al.*, 1998) with or without pathogen according the treatment). The pathogen inoculum adjusted to 10⁵ conidia mL (Fernández *et al.*, 2014). Before applying the spray, the pots were covered with plastic to prevent contact between *B. cinerea* on leaves and T34 on the growth media. The severity of disease was examined 7, 10 and 14 days post-inoculation.

Severity was evaluated using the following score for each leaf: 0 (asymptomatic), 1 (chlorotic spots), 2 (necrotic specks), 3 (necrotic spots), and 4 (dead leaf).

In both studies, perlite from the rhizosphere of the pots with P+T34 (control and inoculated with *B. cinerea*) were collected at 0 and 14 days post-inoculation. Three pots from each treatment were sampled. Populations of T34 were calculated by the method of dilution plate (Johnson & Curl, 1972) in H₂O_d-agar (0,7g Agar L⁻¹ H₂O_d) and by the selective medium Trichoderma (Chung & Hoitink, 1990). Data corresponded to the average of two dilutions performed for each pot and three plates of medium Trichoderma for each dilution were used.

2.4. Statistical analysis

For all studies data was analysed by IBM SPSS Statistics 19 version statistical software and results are expressed as means \pm standard error (SE). The areas of the collection of aligned peaks were studied using standardised PCA to detect differences in the secretion pattern of exudates between treatments. Values of the first and second components were subjected to analysis of variance (ANOVA) ($P < 0.05$). The quantity of known metabolites was analysed by ANOVA ($P < 0.05$) to study the factor treatment. Data from T34 growth rate studies and from growth chamber studies were analysed with ANOVA to study the factor treatment over the evaluation days and to study within treatments the effect of the evaluation days. When significant differences were observed ($P < 0.05$) and more than two groups were present, the Duncan's multiple range test was performed ($P < 0.05$). In cases when normal distribution and homogeneity of variances were not found, the Kruskal-Wallis test was performed ($P < 0.05$).

3. Results

In sterile conditions up to 200 metabolite peaks per chromatogram were detected. The PCA of the peak areas showed that the metabolite composition of root exudates from *B. cinerea* inoculated plants differed from that of non-inoculated control plants (Fig. 1). PCA 1 showed a variability of 52% while that of PCA 2 is 35%. Values from PCA 1 were significantly different ($P < 0.05$) between inoculated and non-inoculated samples.

Peaks of 13 exuded metabolites were identified (Table 1). Significant differences were observed in the concentration of 3 metabolites between plants inoculated and non-inoculated with *B. cinerea*. The concentration of gluconic acid increased by almost 5 times in the presence of *B. cinerea*, which contrasts with the concentrations of inositol and sucrose which decreased by up to 3 and 9 times, respectively.

The growth rates of T34 – measured by the size of the colony radiuses – increased significantly from day 2 to day 4 in the presence of growth media SNA2 and SNA3; this was not the case in the presence of SNA1 (Fig. 2a). Colony radius significantly increased throughout the study period (day 2, 3 and 4).

Over the course of the study period, mycelia of T34 grown in SNA3 were characterised by more defined concentric rings, denser conidial production and higher abundances of pustules than the colonies of T34 grown in SNA1 and SNA2. Moreover, the conidia of T34 grown in SNA3 growth media became greener and darker earlier than in samples grown in SNA1 and SNA2. In terms of pustule abundance only subtle differences were observed between colonies of T34 grown in SNA1 and SNA2, with samples grown in SNA2 having a slightly higher abundance. Fig. 2b-d show representative images of the growth of T34 colonies on day 4.

When the T34 enriched perlite was compared to non-enriched perlite (Fig. 3) in the growth chamber study, disease severity at day 10 and 14 after pathogen inoculation was 12.59 % and 24.34 % lower, respectively. Levels of disease severity in the T34 non-enriched perlite samples increased by 49.21 % after inoculation (day 7, 10 and 14). Disease severity in the T34 enriched perlite samples remained the same from day 10 to 14 after inoculation.

The populations of T34 were unchanged when the pathogen was inoculated on the leaves and decreased significantly – by up to 50 % – in the absence of the pathogen (Fig. 4).

4. Discussion

The fact that the presence of *B. cinerea* can modify the secretion pattern of root exudates is in agreement with the conclusions of previous studies (Hale & Moore, 1979; Lanoue *et al.*, 2010). Other studies have observed that plants exposed to pathogens show an increase in the content of diverse organic acids in root exudates such as succinic acid and malic acid, but not gluconic acid (Kamilova *et al.*, 2006b; Rudrappa *et al.*, 2008). A study by Kamilova *et al.* (2006b) also noted a decrease in sugar content but not in sucrose content. No information in relation to the effect of pathogens on inositol content of root exudates was found.

The closest similarities to our results on metabolite changes were found in botrytised aerial plant organs but not in root exudates. According to Ribéreau-Gayon *et al.* (2006) *B. cinerea* infection leads to the accumulation of gluconic acid in grape tissues which are a characteristic secondary product of sugar degradation when *B. cinerea* emerges

outside of the grape. Other authors have shown, through the metabolic profiling of sunflower cotyledons infected by *B. cinerea*, a 90 % decrease in sucrose and 75 % decrease in inositol 48 h after inoculation (Dulermo *et al.*, 2009). Although these results occurred in aerial organs of the infected plant, we could hypothesize that results in root exudates may reflect the metabolite status in other plant parts depending on factors, such as the type of pathogen involved.

The production of gluconic acid and the subsequent host tissue acidification enhances the establishment of conditions suitable for the necrotrophic development of *Penicillium expansum* (Hadas *et al.*, 2007). De Cal *et al.* (2013) also suggest that ambient pH is linked to pathogenicity processes because *Monilinia fructicola* accumulated gluconic acid at the infection site as the main organic acid. For *B. cinerea* the accumulation of organic acid is also an advantage as it helps reduce the pH, facilitating the degradation of the cell walls of the host by polygalacturonases. In contrast, this function has been traditionally associated with the production of oxalic acid (Elad *et al.*, 2007) rather than gluconic acid.

Some authors report that while gluconic acid provided benefits to certain rhizosphere microorganisms, it is still considered an antifungal agent (Kaur *et al.*, 2006) and the most frequent organic acid produced by rhizobacteria to solubilise insoluble or poorly soluble mineral phosphates (Rodríguez & Fraga, 1999). However, in our study the growth and development of T34 was not negatively affected.

Trichoderma spp. are able to thrive in a wide range of external pH conditions and is more efficient in acidic than in alkaline soils (Benítez *et al.*, 2004). Nevertheless, the optimum pH varies among isolates from pH 4.0 to 6.8 (Steayaert *et al.*, 2010). In SNA3

the pH was more acidic than the other media and that could have affected the growth. However, in SNA2 the pH was the same as in SNA1 and there was a significant T34 growth increase, indicating that gluconic acid has a pH independent growth promoting effect on T34. It should be noted that the dose of gluconic acid used in SNA2 is the same as was obtained in exudates. Lugtenberg *et al.* (2001) reported the crucial role of organic acids in proliferation and root colonisation by BCA *P. fluorescens* WCS365. Moreover, according Zhang *et al.* (2014) some organic acids from cucumber root exudates (oxalic acid, malic acid and citric acid) increase growth and conidial germination of *Trichoderma harzianum* T-E5, which is positive for root colonisation. *B. cinerea* cultured *in vitro* with organic acids (citric or malic acid) also had a higher mycelial production than in media without them (Verhoeff *et al.*, 1988).

T34 colonising roots triggered the induction of systemic resistance, which reduced disease severity significantly as in previous studies (Fernández *et al.*, 2014). The ability of T34 to trigger induced systemic resistance (ISR) against other foliar pathogens in Arabidopsis plants was also demonstrated (Segarra *et al.*, 2009). In addition, a study with cucumber plants has shown that the induction of resistance by T34 can take place via ISR or systemic acquired resistance depending on T34 populations (Segarra *et al.*, 2007). Accordingly, the concentration of T34 in a substrate may play a crucial role in the pathway route of induction of resistance in plants. Therefore, the fact that populations of T34 in the roots were maintained and not decreased in the presence of *B. cinerea* on the leaves could indicate a connection with the reduction of disease severity. Moreover, considering that the presence of *B. cinerea* modifies the secretion pattern of tomato plant exudates, certain compounds within these exudates may stimulate T34 growth. Studies of diverse Trichoderma showed that root exudates of some crops can

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increase the growth of some isolates whereas others have no effect (Jash & Pan, 2007; Bharathi *et al.*, 2008). Exudates secreted by plants provide an environment rich in carbon and energy that can be used by rhizosphere microorganisms and can communicate specifically to them by producing signals that modulate colonisation (Haichar *et al.*, 2014). Furthermore, it is necessary to consider that plants use the ability to recruit microorganisms to obtain the desired benefits of this interaction (Sarma *et al.*, 2015); in this case the benefit might be to help to fend off disease. Accordingly, Pieterse *et al.* (2014) discusses the importance of achieving high population densities to ensure effective ISR. The results obtained in this study indicate the presence of communication from leaf to root via the secretion of exudates that enhance the growth of T34 and in doing so promote resistance to *B. cinerea*.

In conclusion, *B. cinerea* infection on leaves modified the secretion pattern of root exudates in tomato plants. This increased the concentration of gluconic acid and decreased the concentration of sucrose and inositol. This infection also increased populations of T34 and reduced disease severity through induction of systemic resistance. Despite these results, we cannot dismiss the possibility that an unidentified compound from the root exudates could have increased the growth of T34. The results of this study contribute to the body of research in relation to leaf to root communication, the ways in which plants encourage beneficial microorganism relationships, and the role of root exudates in plant defence against foliar pathogens.

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Legends

Figure 1 Principal Component Analysis (PCA 1 and PCA 2) of the peak areas obtained by gas chromatography/mass spectrometry of exuded metabolites from tomato plants inoculated and non-inoculated with *Botrytis cinerea*.

Figure 2 Growth of *Trichoderma asperellum* strain T34 (T34). T34 was grown in Synthetic Nutrient-poor Agar (SNA) with varying sugar compositions: SNA1 (0.2 g L⁻¹ sucrose and 0.2015 g L⁻¹ dextrose), SNA2 (0.2 g L⁻¹ sucrose, 0.2 g L⁻¹ dextrose and 0.0015 g L⁻¹ gluconic acid) and SNA3 (0.2015 g L⁻¹ gluconic acid). (a) Colony radius of T34 from days 2 to 4. Means ± standard error, n= 18 (collected from two separated studies). Different lower case letters show significant differences between treatments and different capital letters show significant differences between days within treatments ($P < 0.05$; Duncan's multiple range test). (b-d) Representative images of T34 growth on day 4 in SNA1, SNA2 and SNA3, respectively.

Figure 3 Disease severity caused by the pathogen *Botrytis cinerea* (10⁵ CFU mL⁻¹) in tomato plants. Plants were grown in two growth media: P, perlite; P+T34, perlite enriched with *Trichoderma asperellum* strain T34 at a concentration of 10⁵ CFU mL⁻¹. Disease was evaluated

at 7, 10 and 14 days post-inoculation. Disease severity was evaluated with a scale of five grades: 0, asymptomatic; 1, chlorotic leaf; 2, necrotic specks; 3, necrotic spot; 4, dead leaf. Means \pm standard error, n= 32 (collected from two separated studies). Different lower case letters show significant differences between treatments and different capital letters show significant differences between days within treatments ($P < 0.05$; ANOVA test and Duncan's multiple range test, respectively).

Figure 4 Populations of *Trichoderma asperellum* strain T34 (T34) calculated by the method dilution plate. Tomato plants were grown in P+T34; perlite enriched with T34 at a concentration of 10^5 CFU mL⁻¹. Two treatments were applied on day 0: P+T34; without Pathogen, P+T34+BC; inoculated with the pathogen *Botrytis cinerea* (10^5 CFU mL⁻¹). Samples from rhizosphere were collected at 0 and 14 days post-inoculation. Means \pm standard error, n= 12 (collected from two separated studies). Different lower case letters show significant differences between treatments and different capital letters show significant difference between days within treatments ($P < 0.05$; ANOVA test).

Table 1 Metabolites composition ($\mu\text{g plant}^{-1}$) of root exudates of tomato seedlings grown on sand in the absence and presence of the pathogen *Botrytis cinerea* (10^5 CFU mL). Means \pm standard error, n=6 (from two separated studies). * indicate significant difference between treatments ($P < 0.05$; ANOVA test).

Metabolites	Control	<i>Botrytis cinerea</i>
arabinose	0.62 \pm 0.06	1.17 \pm 0.27
fructose	0.29 \pm 0.03	0.26 \pm 0.04
galactaric acid	0.83 \pm 0.35	1.29 \pm 0.05

galactose	0.91 ± 0.24	1.42 ± 0.44
gluconic acid	0.30 ± 0.08	1.48 ± 0.17 *
glucose	0.15 ± 0.04	0.08 ± 0.03
inositol	3.82 ± 0.11	1.14 ± 0.14 *
maltose	0.69 ± 0.51	0.60 ± 0.08
mannitol	0.32 ± 0.05	0.20 ± 0.03
rhamnose	1.52 ± 0.05	1.93 ± 0.98
ribose	1.32 ± 0.69	0.56 ± 0.03
sucrose	1.30 ± 0.20	0.14 ± 0.03 *
xylose	0.10 ± 0.03	0.04 ± 0.01







