Effects of the Tomato Pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* and of the Biocontrol Bacterium *Pseudomonas fluorescens* WCS365 on the Composition of Organic Acids and Sugars in Tomato Root Exudate

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The effects of the pathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici* and of the bacterial biocontrol strain *Pseudomonas fluorescens* WCS365, and of both microbes, on the amounts and composition of root exudate components of tomato plants grown in a gnotobiotic stonewool substrate system were studied. Conditions were selected under which introduction of *F. oxysporum* f. sp. *radicis-lycopersici* caused severe foot and root rot, whereas inoculation of the seed with *P. fluorescens* WCS365 decreased the percentage of diseased plants from 96 to 7%. This is a much better disease control level than was observed in potting soil. Analysis of root exudate revealed that the presence of *F. oxysporum* f. sp. *radicis-lycopersici* did not alter the total amount of organic acids, but that the amount of citric acid decreased and that of succinic acid increased compared with the nontreated control. In contrast, in the presence of the *P. fluorescens* biocontrol strain WCS365, the total amount of organic acid increased, mainly due to a strong increase of the amount of citric acid, whereas the amount of succinic acid decreased dramatically. Under biocontrol conditions, when both microbes are present, the content of succinic acid decreased and the level of citric acid was similar to that in the nontreated control. The amount of sugar was approximately half that of the control sample when either one of the microbes was present alone or when both were present. Analysis of the interactions between the two microbes grown together in sterile tomato root exudate showed that WCS365 inhibited multiplication of *F. oxysporum* f. sp. *radicis-lycopersici*, whereas the fungus did not affect the number of CFU of the bacterium.

Tomato foot and root rot (TFRR) is a disease caused by the pathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici*. The disease occurs on tomato in both the greenhouse and the field; it causes significant losses in tomato production and has been reported in at least 32 countries (Jones et al. 1991). TFRR cannot be treated efficiently with chemicals. The use of biocontrol bacteria isolated from the rhizosphere is a promising alternative to prevent the disease. Root exudate compounds such as organic acids, sugars, and amino acids are easily utilized

able nutrients for rhizosphere microorganisms (Kamilova et al. 2006; Rovira 1969; Vancura and Hovadik 1965). The composition of metabolites secreted by germinating seed and by roots is dependent on plant species, plant age, root zone, the plant's mineral nutrition, stress effects, and other environmental factors (Baudoin et al. 2002; Griffiths et al. 1999; Kamilova et al. 2006; Maloney et al. 1997; Yang and Crowley 2000). The exudate composition largely influences the development and composition of the root-associated microflora (Walker et al. 2003). Organic acids are the major tomato root exudate carbon source used by the biocontrol bacterium *P. fluorescens* WCS365. Citric acid is the most abundant organic acid in tomato root exudate (Kamilova et al. 2006; Lugtenberg et al. 2001). Sugars also are present in tomato root exudate but in lower amounts than organic acids (Lugtenberg et al. 2001). Consistent with that observation is the finding that utilization of organic acids is crucial for proliferation and colonization of *P. fluorescens* WCS365 on the roots (De Weert et al. 2002; Lugtenberg et al. 2001), whereas utilization of sugars does not play a significant role in root colonization (Lugtenberg et al. 1999).

The bacterium *P. fluorescens* WCS365 is attracted to the root by exudate chemicals, especially by citric acid and malic acid (De Weert et al. 2004). Subsequently it attaches to the root, where it forms biofilms (Bloembergen et al. 1997, 2004; Chin-A-Woeng et al. 1997). The strain controls TFRR by the mechanism "induced systemic resistance" (ISR) (Kamilova et al. 2005).

In modern horticulture, a strong tendency exists to grow tomato on stonewool substrate. Economic advantages of the use of stonewool are higher crop yield, better control of growth, energy conservation, and independence of soil quality (van Os 1999).

It appeared that growth on stonewool strongly increases exudation compared with growth on a neutral substrate such as glass beads (Kamilova et al. 2006). Also, on stonewool, the organic acid fraction remains the major carbon source and citric and succinic acids remain the most dominant representatives of this fraction (Kamilova et al. 2006).

New stonewool practically does not contain microorganisms (Postma et al. 2000, 2005). It seems reasonable to predict that this provides a biocontrol agent on the seed with an enormous advantage because, in the beginning, when the seed are most vulnerable to *F. oxysporum* f. sp. *radicis-lycopersici* (F. Kamilova, *unpublished*) it does not have to compete with an indigenous microflora. On the other hand, the absence of this

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microflora also provides an advantage for incoming plant pathogens such as *F. oxysporum* f. sp. *radicis-lycopersici*. In the experiments described in the present article, we mimic such conditions. The results indicated that it is possible to efficiently control TFRR in stonewool substrate immersed in commercial plant nutrient solution. We subsequently analyzed the fate of exudate organic acids and sugars during incubation with the two microbes, both individually and under biocontrol conditions. To facilitate interpretation of the results, we did the same analysis in sterile exudates in the absence of the plant. Moreover, we followed the viable counts of the two microbes upon joint incubation in this sterile exudate. The results are described in this article.

RESULTS

Effect of biocontrol bacterium *P. fluorescens* WCS365 on TFRR disease symptoms and plant dry weight of tomato grown in stonewool in the presence of *F. oxysporum* f. sp. *radicis-lycopersici* spores.

In all experiments described in this article, tomato cv. Carmello plants were grown in a sterile system of glass beakers

Table 1. Influence of *Pseudomonas fluorescens* WCS365 on the development of tomato disease symptoms and on tomato plant dry weight^a

Microbes present	Diseased plants (%)	Dry weight (mg/plant)
None	0	2.4 ± 0.1
<i>Fusarium</i>	96	1.9 ± 0.1
WCS365	0	2.5 ± 0.1
<i>Fusarium</i> + WCS365	7	1.9 ± 0.3

^a Influence of the biocontrol bacterium *Pseudomonas fluorescens* WCS365 on the development of tomato foot and root rot disease symptoms and on the dry weight of tomato plant growing in stonewool containing spores of the tomato pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Fusarium*)

containing a layer of stonewool saturated with plant nutrient solution (PNS). It appeared to be easy to cause disease symptoms of TFRR by *F. oxysporum* f. sp. *radicis-lycopersici* as well as to control the disease by using the biocontrol bacterium *P. fluorescens* WCS365. Despite the very high disease pressure, the presence of the biocontrol strain decreased the number of brownish lesions to a very low level (Table 1). Disease correlated with a decrease of plant fresh weight of approximately 25%. The presence of the biocontrol strain prevented this negative effect of the pathogen. The presence of the biocontrol strain alone had no significant effect on plant dry weight (Table 1).

Effects of pathogen and biocontrol agent on the root exudate composition of tomato plants grown on stonewool.

The organic acid and sugar fractions of root exudates of plants grown gnotobiotically on stonewool was compared with that of plants incubated with the pathogen and the biocontrol agent alone, as well as of plants grown in the presence of both microbes (Tables 2 and 3). Citric acid and succinic acid appeared to be the major organic acids in tomato root exudate of 10-day-old plants (Table 2). The presence of *F. oxysporum* f. sp. *radicis-lycopersici* reduced the amount of citric acid to approximately 60% and increased the amount of succinic acid approximately two- to threefold. In contrast, the presence of *P. fluorescens* WCS365 resulted in a 50% increase of the citric acid level and in a fivefold reduction of succinic acid (Table 2). Under biocontrol conditions (i.e., in the presence of both microbes), the level of citric acid increased and that of succinic acid decreased (Table 2).

Fructose and glucose appeared to be the major root exudate sugars in the absence of microbes. The presence of the pathogen substantially reduced the levels of both sugars. The presence of the biocontrol microbe hardly affected the level of fructose but resulted in the disappearance of glucose. When both

Table 2. Organic acid composition of root exudates of tomato grown on stonewool in the absence and presence of the pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Fusarium*) or the biocontrol bacterium *Pseudomonas fluorescens* WCS365^a

Acid	Amount of organic acid (µg/plant) in the absence and presence of microbes							
	Control		<i>Fusarium</i>		WCS365		<i>Fusarium</i> + WCS365	
	Amount	Total (%) ^b	Amount	Total (%)	Amount	Total (%)	Amount	Total (%)
Citric	33.5 ± 3.7	77.7	20.6 ± 1.8	48.7	50.4 ± 4.3	95.6	39.1 ± 5.0	94.9
Malic	0.75 ± 0.1	1.7	ND	...	ND	...	ND	...
t-Aconitic	0.1 ± 0.01	0.2	0.04 ± 0.02	0.1	0.14 ± 0.02	0.3	0.15 ± 0.01	0.4
Succinic	7.95 ± 2.12	18.5	20.5 ± 1.7	48.5	1.5 ± 0.5	2.8	1.4 ± 0.7	3.4
Fumaric	0.11 ± 0.01	0.3	0.11 ± 0.02	0.3	0.08 ± 0.01	0.2	0.08 ± 0.01	0.2
Pyroglutamic	0.71 ± 0.24	1.6	1.0 ± 0.1	2.4	0.58 ± 0.1	1.1	0.51 ± 0.1	1.1
Total amount	43.12	100	42.30	100	52.70	100	41.20	100

^a Exudate of 60 plants in a volume of 30 ml was used in each incubation; ND = not detected (i.e., lower than detection limit of 0.001 µg/plant).

^b Percentage of total organic acid.

Table 3. Sugar composition of root exudates of tomato grown on stonewool in the absence and presence of the pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Fusarium*) or the biocontrol bacterium *Pseudomonas fluorescens* WCS365^a

Sugar	Amount of sugar (µg/plant) in the absence and presence of microbes							
	Control		<i>Fusarium</i>		WCS365		<i>Fusarium</i> + WCS365	
	Amount	Total (%) ^b	Amount	Total (%)	Amount	Total (%)	Amount	Total (%)
Ribose	0.41 ± 0.14	3.0	0.32 ± 0.06	3.9	0.11 ± 0.03	1.6	0.20 ± 0.04	2.4
Xylose	ND	...	0.50 ± 0.15	6.0	ND	...	0.30 ± 0.07	4.0
Fructose	7.50 ± 0.60	54.8	4.15 ± 0.54	50.3	6.10 ± 1.46	89.3	4.65 ± 0.7	57.0
Glucose	4.68 ± 0.62	34.2	2.64 ± 0.34	32.0	ND	...	2.10 ± 0.30	25.6
Maltose	1.10 ± 0.12	8.0	0.64 ± 0.20	7.8	0.62 ± 0.09	9.1	0.90 ± 0.20	11.0
Total amount	13.70	100	8.25	100	6.83	100	8.20	100

^a Exudate of 60 plants in a volume of 30 ml was used in each incubation; ND = not detected (i.e., lower than detection limit of 0.001 µg/plant).

^b Percentage of total sugar. Root exudates were collected after incubation of seed for 10 days at 21 to 24°C.

microbes were present in the system, glucose appeared again in the exudate, and it led to an increase of the total amount of detected sugars compared with the sample where only *P. fluorescens* WCS365 was present. The presence of both microbes resulted in the same effect as the presence of the pathogen alone (Table 3).

Effect of pathogen and biocontrol agent on the composition of sterile tomato root exudate in the absence of plant.

To evaluate the ability of the added microbes to utilize tomato root exudate organic acids and sugars without interference of the plant, we cultured them in the absence of the plant. Sterile tomato root exudate contains citric and succinic acids as the major organic acids. Incubation of sterile root exudate with either *F. oxysporum* f. sp. *radicis-lycopersici* or WCS365, or with both microbes, reduced the amount of total organic acid by more than 90%. Citric acid levels were almost 10-fold reduced and succinic acid became undetectable (Table 4).

Sterile tomato root exudate contains, in molar ratio, 10-fold less sugar (Table 5) than organic acid (Table 4). It contains fructose and glucose as the major sugars (Table 5). Incubation with either the pathogen *F. oxysporum* f. sp. *radicis-lycopersici* or with the biocontrol agent *P. fluorescens* WCS365, or with both microbes, reduced the total amount of sugar by more than 75%. Most of the fructose and glucose disappeared under all conditions (Table 5). It should be noted that the sugar arabinose appeared upon incubation of root exudate with either microbe, especially when *F. oxysporum* f. sp. *radicis-lycopersici* was present.

Growth of microbes in sterile tomato root exudate.

Upon incubation of *P. fluorescens* WCS365 in sterile tomato root exudate, the number of CFU increased from 10^5 to 10^8

CFU/ml during the first 24 h and did not change in the next 48 h. After testing growth of WCS365 in root exudate, we examined bacterial growth on the individual major components of tomato exudates (citric and succinic acids; fructose, and glucose) as the sole carbon sources. In PNS supplemented with succinate (0.3 mM, the approximate concentration found in tomato root exudate), *P. fluorescens* WCS365 also reached the stationary phase within 24 h; whereas, with citrate (0.3 mM) as a sole carbon source, the number of CFU initially decreased approximately fourfold (Fig. 1A). After 48 to 72 h, the numbers of bacteria in both media were similar. The presence of *F. oxysporum* f. sp. *radicis-lycopersici* did not influence the numbers of CFU of WCS365 in any of the tested media (Fig. 1A). Growth of WCS365 in media containing either glucose (0.3 mM) or fructose (0.3 mM) was similar and the highest number of bacteria did not exceed 5×10^7 CFU/ml. The number of *F. oxysporum* f. sp. *radicis-lycopersici* propagules increased from 10^5 to 5×10^5 to 10^6 propagules/ml when the fungus was cultivated alone in any of the tested media. In contrast, when, in addition, *P. fluorescens* WCS365 was present in tomato root exudate or in PNS supplemented with either citric or succinic as a sole carbon source, the number of propagules stayed at the level of inoculation or even decreased slightly (Fig. 1B). A similar effect was observed upon cultivation of microbes in PNS supplemented with glucose or fructose (data not shown).

DISCUSSION

The levels of the compounds detected in root exudate in the presence of microbes are the net result of a number of complex processes, which include exudation of compounds by roots and microbes and utilization of these nutrients by these organisms. Moreover, interactions between the plant and the mi-

Table 4. Effects of incubation of sterile tomato root exudate with the pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Fusarium*) or the biocontrol bacterium *Pseudomonas fluorescens* WCS365 on the organic acid composition^a

Organic acid	Amount of organic acid (µg/plant)							
	Control		<i>Fusarium</i>		WCS365		<i>Fusarium</i> + WCS365	
	Amount	Total (%) ^b	Amount	Total (%)	Amount	Total (%)	Amount	Total (%)
Citric	27.8	68.9	3.43 ± 0.2	88.9	2.9 ± 0.2	91.0	2.66 ± 0.09	89.0
Pyruvic	ND	...	ND	...	0.1 ± 0.03	3.1	ND	...
Malic	0.5	1.2	ND	...	ND	...	ND	...
t-Aconitic	0.01	0.03	ND	...	ND	...	ND	...
Succinic	11.2	27.7	ND	...	ND	...	ND	...
Fumaric	0.06	0.15	0.01 ± 0.002	0.3	0.007 ± 0.002	0.2	Trace	...
Pyroglutamic	0.8	2.0	0.42 ± 0.08	10.8	0.18 ± 0.08	5.7	0.33 ± 0.09	11.0
Total amount	40.37	100	3.86	100	3.19	100	3.00	100

^a Exudate of 60 plants in a volume of 30 ml was used in each incubation; ND = not detected (i.e., lower than detection limit of 0.001 µg/plant); Trace = an amount lower than 0.005 µg/plant.

^b Percentage of total organic acid.

Table 5. Effects of incubation of sterile tomato root exudate with the pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Fusarium*) or the biocontrol bacterium *Pseudomonas fluorescens* WCS365 on the sugar composition^a

Sugar	Amount of sugar (µg/plant)							
	Control		<i>Fusarium</i>		WCS365		<i>Fusarium</i> + WCS365	
	Amount	Total (%) ^b	Amount	Total (%)	Amount	Total (%)	Amount	Total (%)
Ribose	0.05	1.3	0.04 ± 0.01	4.5	0.04 ± 0.01	6.2	ND	...
Xylose	0.02	0.5	ND	...	ND	...	ND	...
Arabinose	ND	...	0.56 ± 0.06	62.9	0.19 ± 0.02	29.2	0.63 ± 0.03	67.8
Fructose	2.70	69.2	0.12 ± 0.01	13.5	0.13 ± 0.01	20.0	0.11 ± 0.02	11.8
Glucose	0.82	21.0	0.17 ± 0.01	19.1	0.13 ± 0.01	20.0	0.19 ± 0.01	20.4
Maltose	0.31	8.0	ND	...	0.16 ± 0.02	24.6	ND	...
Total amount	3.90	100	0.89	100	0.65	100	0.93	100

^a Exudate of 60 plants in a volume of 30 ml was used in each incubation. This exudates batch was identical to the one used in Table 4. ND = not detected (i.e., lower than detection limit 0.001 µg/plant).

^b Percentage of total sugar.

crobes as well as between the microbes make the picture even more complex.

In new stonewool substrate, biocontrol strain *P. fluorescens* WCS365 reduced the disease pressure caused by *F. oxysporum* f. sp. *radicis-lycopersici* from 96 to 7% (Table 1). Apparently, both infection by *F. oxysporum* f. sp. *radicis-lycopersici* and biocontrol by *P. fluorescens* WCS365 can be very efficient in this system compared with a potting soil system. In a typical biocontrol experiment in the latter substrate, the disease pressure drops from 70 to 40% (Dekkers et al. 2000; Kamilova et al. 2005). This effect presumably is due to the lack of competition by indigenous microbes in the sterile system. The efficient biocontrol result in stonewool (Table 1) is very promising for the application of biocontrol agents coated on seed sown in new stonewool mats; first, because germinating seed are much more vulnerable to *F. oxysporum* f. sp. *radicis-lycopersici* than older plants (F. Kamilova, *unpublished*) and, second, because new mats are practically sterile and, therefore, the biocontrol agent initially will be the dominant microbe. This optimistic expectation was further confirmed when biocontrol was carried out in commercially available stonewool plugs (Validov et al. in press).

The presence of relatively high amounts of the organic acids citric acid and succinic acid (Table 2) and of the sugars fructose and glucose (Table 3) in tomato root exudate of tomato grown in stonewool confirmed results of previous experiments (Kamilova et al. 2006). The presence of the microbes increased the exudation by the plant, as can be concluded from the increased levels of succinic acid in the presence of *F. oxysporum* f. sp. *radicis-lycopersici* and of citric acid in the presence of *P. fluorescens* WCS365 (Table 2). It is possible that exudation of other components also was stimulated but that these compounds were not detected because they were consumed or degraded by the organisms. Surprisingly, the 50% increase in citric acid levels in root exudate had no influence on the plant's dry weight (Table 1). Furthermore, both microbes seem to utilize some organic acids; however, they clearly have a different preference for different organic acids (Table 2).

The presence of the microbes reduced the total amount of sugars in root exudate (Table 3). The reduction of glucose in the presence of *P. fluorescens* WCS365 was especially dramatic (Table 2). No evidence for an increase in exudation of sugars due to the presence of the microbes was found.

Fig. 1. Fate of **A**, *Pseudomonas fluorescens* WCS365 and **B**, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl) grown in plant nutrient solution supplemented with tomato root exudate (pH 5.5), citrate (0.3 mM, pH 5.5), or succinate (0.3 mM, pH 5.5). Results are the mean of duplicate samples. Vertical bars indicate the standard deviation.

The results observed in the presence of three organisms, the plant and two microbes, are hard to interpret because all three organisms can play an active role in synthesis, secretion, or utilization of exudate components. Therefore, we also simplified the system somewhat by incubating the microbes in sterile exudate in the absence of the plant. The results showed that both microbes can utilize approximately 80% of the fructose and glucose from exudates (Table 5). Similarly, the microbes consumed large amounts of citric acid and even whole succinic acid (Table 4). In separate experiments, it was shown that succinic acid is consumed faster by WCS365 than citric acid (Fig. 1A).

Incubation of exudate with each of the microbes individually, especially with *F. oxysporum* f. sp. *radicis-lycopersici*, resulted in the appearance of arabinose in exudate to such an extent that it became the major sugar (Table 5). The appearance of arabinose can be explained by secretion of microbial enzymes which degrade arabinose-containing oligomers or polymers of the plant present in exudates (Timotiwi and Sakurai 2002). Comparison of Tables 3 and 5 shows that, in the presence of the plant, arabinose is absent, suggesting that, if arabinose also is formed under those conditions, it is taken up by the plant.

P. fluorescens WCS365 is known as an agent acting through ISR in *Arabidopsis thaliana* (Gerrits and Weisbeek 1996) and in tomato (Kamilova et al. 2005). The strong negative effect of this bacterium on the viable counts of *F. oxysporum* f. sp. *radicis-lycopersici* in the experiments when both microbes were cultivated together in isolated root exudate and in PNS containing one of the major organic acids (Fig. 1B) and sugars (data not shown) present in exudates indicated inhibition of multiplication of the fungus by WCS365. This observation, together with data showing colonization of hyphae by *P. fluorescens* WCS365 in vivo (Bolwerk et al. 2003; De Weert et al. 2004) and in vitro (De Weert et al. 2004) indicates that, in addition to the mechanism of induction of systemic resistance, this strain also contributes to controlling the disease by attacking *F. oxysporum* f. sp. *radicis-lycopersici* directly. However, this effect may not be a specific trait for one or more biocontrol strains but could be a general characteristic of many rhizosphere microbes, which are known to have a buffering effect on disease suppression in stonewool. It was shown that cucumber plants grown on used stonewool slabs are more resistant toward *Pythium* spp. infection than plants grown on new or autoclaved used slabs (Postma et al. 2000, 2005). Despite the elaborate methods of analysis, the authors did not find the agent of disease suppression. They drew the conclusion that microflora developed during plant growth decreased the possibility for the pathogenic agent to reach and proliferate in the root system due to various factors, but mainly due to competition (Postma et al. 2000, 2005).

MATERIALS AND METHODS

Microbial strains and growth conditions.

Biocontrol strain *P. fluorescens* WCS365 was grown under vigorous aeration in 20-fold-diluted tryptic soy broth (1/20 TSB; Difco Laboratories, Detroit, U.S.A.) at 28°C. *F. oxysporum* f. sp. *radicis-lycopersici* was cultivated routinely on potato-dextrose agar (PDA) (Difco Laboratories) or in Czapek-Dox liquid medium (Difco Laboratories) at 28°C with vigorous shaking. Growth of microbes in sterile tomato root exudate was performed as follows. Samples of 5 ml of sterile tomato root exudate were inoculated either with cells of *P. fluorescens* WCS365 at a final concentration 10^5 CFU/ml, with spores of *F. oxysporum* f. sp. *radicis-lycopersici* at a final concentration of 10^4 spores/ml, or with both microbes. The suspensions sub-

sequently were incubated at 28°C with vigorous aeration. The effect of individual carbon sources on the growth of the microbes was evaluated by following the number of CFU upon growth in PNS supplemented with citrate, succinate, glucose, or fructose at concentrations 0.3 mM and a pH value of 5.5. When PNS was supplemented with organic acid, the pH was adjusted with 2 N NaOH. *P. fluorescens* strain WCS365 was pregrown in sterile tomato root exudate for 3 days, washed twice with sterile phosphate-buffered saline (PBS), and inoculated in PNS supplemented with either tomato root exudate or citric acid, succinic acid, fructose, or glucose as a sole carbon source to a final concentration 10^5 CFU/ml. CFU of bacterial cells and of fungal propagules were determined by dilution plating, for the bacterium on King's medium B (King et al. 1954) solidified with 1.8% agar (Select Agar, Gibco BRL, Life Technologies, Paisley, UK) and supplemented with cycloheximide (100 µg/ml); and for fungi on PDA supplemented with kanamycin (50 µg/ml). Each sample was analyzed in duplicate and each experiment was repeated at least three times.

Plant growth and preparation and analysis of sterile exudate.

Sterile root exudate was prepared from tomato cv. Carmello and analyzed as described previously (Kamilova et al. 2006), with the minor modification that commercial PNS (PPO, Naaldwijk, The Netherlands) was used in the present work. The same exudate samples were used for the analysis of organic acids and of sugars. Therefore, the method of sample preparation was identical for organic acid and sugar analysis. All samples were prepared in triplicate.

Biocontrol of TFRR in sterile stonewool.

An overnight culture of *P. fluorescens* WCS365 grown in 1/20 TSB was spun down, washed twice in sterile PBS, and added to PNS to a final concentration of 10^7 CFU/ml. Spores of *F. oxysporum* f. sp. *radicis-lycopersici* were prepared according Chin-A-Woeng and associates (1998), washed twice in PBS, and added to PNS to a final concentration of 10^2 spores/ml. The layer of 5 g of stonewool (Rockwool, Brinkman Agro BV, 's-Gravenzande, The Netherlands) was placed on the bottom of a 400-ml glass beaker and autoclaved for 15 min at 120°C. Subsequently, this stonewool was saturated with 60 ml of sterile PNS. Tomato seed were sterilized and sown as described previously (Kamilova et al. 2006). Each sample consisted of three replicates containing 30 plants each. After 10 days of growth, root exudates were collected and plant roots were examined for the presence of disease symptoms. Plants which showed brownish lesions were scored as sick. The biocontrol experiment was performed twice.

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