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RESEARCH ARTICLE

Agrobacterium tumefaciens C58 presence affects *Bacillus velezensis* 32a ecological fitness in the tomato rhizosphere

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Abstract

The persistence of pathogenic *Agrobacterium* strains as soil-associated saprophytes may cause an inconsistency in the efficacy of the biocontrol inoculants under field condition. The study of the interaction occurring in the rhizosphere between the beneficial and the pathogenic microbes is thus interesting for the development of effective biopesticides for the management of crown gall disease. However, very little is still known about the influence of these complex interactions on the biocontrol determinants of beneficial bacteria, especially *Bacillus* strains. This study aimed to evaluate the effect of the soil borne pathogen *Agrobacterium tumefaciens* C58 on root colonization and lipopeptide production by *Bacillus velezensis* strain 32a during interaction with tomato plants. Results show that the presence of *A. tumefaciens* C58 positively impacted the root colonization level of the *Bacillus* strain. However, negative impact on surfactin production was observed in *Agrobacterium*-treated seedling, compared with control. Further investigation suggests that these modulations are due to a modified tomato root exudate composition during the tripartite interaction. Thus, this work contributes to enhance the knowledge on the impact of interspecies interaction on the ecological fitness of *Bacillus* cells living in the rhizosphere.

Keywords Rhizosphere colonization · Lipopeptide production · Interspecies interaction · *Bacillus velezensis* 32a · *Agrobacterium tumefaciens* C58 · Biological control

Introduction

Members of *Bacillus* genus living in association with plant roots are among the most efficient microbial biocontrol agents used for the management of plant diseases. These strains, more particularly those belonging to *Bacillus velezensis*, are characterized by high rhizosphere competence and huge genetic equipment devoted to the production of a wide range of structurally different bioactive metabolites (Qiao et al. 2014; Chowdhury et al. 2015; Hossain et al. 2015). Of these later, lipopeptides belonging to the surfactin, iturin, and fengycin families are of high importance. These compounds, readily secreted under in vitro conditions, are also the main antimicrobial compounds produced at significant levels under natural growth conditions (Debois et al. 2014; Cawoy et al. 2015). When produced, lipopeptides play a key role in the tritrophic interaction occurring between the beneficial *Bacillus* strain, the host plant, and the phytopathogen (Raaijmakers et al. 2010). They can facilitate the colonization of plant roots by the producing bacterium, act as antagonists by directly inhibiting plant pathogens, and stimulate the host plant immunity to increase resistance towards further pathogen attack (Ongena and Jacques 2008; Mnif and Ghribi 2015). This wide range of activities makes the *Bacillus* producing strains among the most efficient biocontrol agents of plant diseases (Cawoy et al. 2011). However, the success of these bioproducts globally suffers from some inconsistencies in their efficacy under greenhouse or field conditions (Debois et al. 2014). In fact, the

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growth under laboratory-controlled conditions in a rich medium is quite different from that on a nutrient-excreting root surface. Rhizosphere specific factors (nutritional status, growth rate, oxygen availability, biofilm formation), environmental conditions (temperature, pH, drought), and interactions occurring between the soil-inhabiting microbes may affect the growth and production of lipopeptides by *Bacillus* strains, thus influencing their biocontrol efficacy. Understanding which and how the complex rhizosphere factors may modulate the production of lipopeptides is a crucial point to improve the efficacy of *Bacillus* biocontrol agents in reducing plant diseases.

The impact of several abiotic factors inherent to the rhizosphere on the production of lipopeptides has been addressed in previous works (Nihorimbere et al. 2009, 2012; Pertot et al. 2013). However, little is known about the effect of biotic factors, such as the presence of soil-borne pathogens, on the biosynthesis of these key biocontrol metabolites (Zihalirwa Kulimushi et al. 2017). Among the telluric plant pathogens, *Agrobacterium tumefaciens* is a Gram-negative bacterium causing crown gall tumors on a wide range of plants (Hammami et al. 2009). This disease causes severe annual losses to growers and nursery men worldwide due to unsalable nursery stock, low productivity from galled trees, and increased susceptibility of infected plants to other pathogens and to environmental stress (Bliss et al. 1999). In the European countries, the crown gall agent is considered a quality pathogen and a quarantine pathogen in other countries. In Tunisia, the Ministry of Agriculture authorizes trade of nursery productions only if they bear less than 1% visible galls (Rhouma et al. 2008). A strict sanitary control of imported propagating material for the presence of crown gall has been enforced in the country. Nevertheless, in spite of the prophylactic measures implemented to control the disease, several Tunisian nurseries proved to be contaminated.

The failure in crown gall control may be attributed to several factors, including inoculum transfer from the soil (Yakabe et al. 2010). Due to their ability to form biofilms, pathogenic agrobacteria can survive in bulk soil for long period of time, where they could persist as soil-associated saprophytes (Abarca-Grau et al. 2011). They can be detected even after 16 years of the removal of infected plants (Krimi et al. 2002). Infested soil can be thus a risk for disease re-occurrence during further planting of susceptible hosts. Studying the interaction occurring in the rhizosphere between the beneficial and the pathogenic microbes seems to be interesting for the development of effective biopesticides in the management of crown gall disease.

In previous works, we reported that *B. velezensis* strain 32a was an efficient crown gall biocontrol agent co-producing surfactin, iturin, and fengycin lipopeptides under laboratory conditions (Ben Abdallah et al. 2015; Frikha-Gargouri et al. 2017; Ben Abdallah et al., 2018a, b). In this study, the first aim was to evaluate the impact of interaction between *A. tumefaciens* C58 and *B. velezensis* 32a, in the tomato rhizosphere, on 32a root colonization level and its lipopeptide production. For this purpose, strain 32a was applied in tomato rhizosphere in presence or absence of the bacterial phytopathogen *A. tumefaciens*. The 32a population density in tomato roots and the amount of lipopeptides produced under these conditions were determined and compared. To explain the observed changes occurring during the interaction between the two microbes *in planta*, we also investigated to what extent the 32a growth and lipopeptide synthesis may be modulated by the nutritional status imposed by the plant.

Materials and methods

Bacterial strains and plant material

The *B. velezensis* strain 32a and the bacterial pathogen *A. tumefaciens* strain C58 were used in this study. They were routinely grown in Luria-Bertani (LB) agar at 30 °C and maintained at 4 °C before use. For long-term storage, *Agrobacterium* and *Bacillus* strains were kept at − 80 °C using glycerol 15 and 30%, respectively.

Tomato seeds cv. Rio Grande were used for in vivo experiments. They were grown on Long Ashton nutrient solution (Abdelly et al. 1995) and incubated at 23 ± 1 °C in a culture room with a 16 h light/8 h dark photoperiod.

Natural and recomposed tomato root exudate preparation

Tomatoes seeds were surface sterilized by dipping them in 70% (v/v) ethanol for 3 min followed by 5% sodium hypochlorite for 5 min, and they were then rinsed three times with sterile distilled water. Seeds were allowed to germinate on sterile filter paper in Petri dishes in the dark at 25 °C. After 96 h, uniform seedlings were transferred in 50-mL tubes filled with Long Ashton nutrient solution (Abdelly et al. 1995) and incubated at 23 ± 1 °C in a culture room with a 16 h light/8 h dark photoperiod. Root exudates produced after 21 days were collected and three times vacuum concentrated. The natural exudates (NE) were used after filtration through 0.22 μm membrane (Milipore).

The tomato recomposed exudate (RE) medium was prepared according to Nihorimbere et al. (2012). It contained the following: $(NH_4)_2SO_4$·2 g L^{-1}, yeast extract 1 g L^{-1}, K_2HPO_4 1 g L^{-1}, KCl 0.5 g L^{-1}, $MgSO_4$ 7 H_2O 0.5 g L^{-1}, $CuSO_4$ 1.6 mg L^{-1}, $Fe_2(SO_4)_3$ 1.2 mg L^{-1}, $MnSO_4$ 0.4 mg L^{-1}, glucose 0.8 g L^{-1}, fructose 1.3 g L^{-1}, maltose 0.2 g L^{-1}, ribose 0.02 g L^{-1}, citrate 5.6 g L^{-1}, succinate 1.4 g L^{-1}, malate 0.2 g L^{-1}, fumarate 0.2 g L^{-1}, casamino acids 0.5 g L^{-1}.

Biomass and lipopeptide production upon growth in natural and recomposed tomato root exudates

The bacterial suspensions were prepared from 16 h old cultures previously grown at 25 °C in LB broth. Harvested cells were resuspended in NaCl 0.9% and used to inoculate the natural and the recomposed exudates at a final concentration of 1×10^7 CFU mL^{-1}. *B. velezensis* strain 32a cultures, co-inoculated or not with *A. tumefaciens* C58, were incubated with continuous shaking for 72 h at 25 °C. After incubation, the bacterial biomass was determined by plate count on LB medium. The supernatants obtained after centrifugation were filtered through a 0.22 μm membrane and processed for lipopeptide purification and quantification. Each experiment was repeated three times.

Root colonization and lipopeptide production by *B. velezensis* strain 32a during interaction with tomato seedlings

Tomato seedlings were obtained from surface sterilized and pre-germinated seeds as described above. They were inoculated with *B. velezensis* 32a or a combination of both *B. velezensis* 32a and *A. tumefaciens* C58 (strains were inoculated at a final concentration of 1×10^7 CFU mL^{-1} in the nutrient solution) and grown aseptically in a culture room for 1 month. Non inoculated tomato seedlings were used as control. Every 6 days, tomato root colonization and lipopeptide production by strain 32a were determined. The 32a cells inhabiting the rhizosphere were quantified using the root samples, after vortexing for 20 min in physiological solution containing 0.05% (w/v) Tween 20. The resulting solutions were serially diluted, and the cell concentrations were determined by plate count on LB medium after 24 h of incubation at 30 °C, on the basis of typical morphology of the colonies. Physiological solution without Tween 20 was used as control to check the effect of 0.05% (w/v) Tween 20 on the growth of *B. velezensis* 32a. The absence of growth inhibition effect was verified by comparing the 32a cell concentration in physiological solution containing 0.05% (w/v) Tween 20 with that of control.

To evaluate the production of lipopeptides in the hydroponic cultures, root samples were immersed in 6 mL acetonitrile/formic acid 0.1% and vortexed for 5 min in the presence of glass beads. They were then incubated overnight at 30 °C with agitation at 140 rpm. The extract was centrifugated and the supernatant was combined with the hydroponic liquid collected at the same time. The combined solution was concentrated, filtered through a 0.22 μm membrane, and processed for analysis of lipopeptide content. Each experiment was repeated three times.

Lipopeptide purification and quantification

To evaluate the production of lipopeptides, the filtrates obtained were firstly submitted to a solid phase extraction on a C18 cartridge (1 g; Alltech Maxi-Clean). Samples were added to columns appropriately conditioned, and the loaded material was washed with MilliQ water before the elution of the lipopeptides with pure methanol. The solutions were vacuum dried (Speed Vac Plus, SC 110A, Savant, GMI, Ramsey, USA) and redissolved in 200 μL of methanol.

The resulting samples were analyzed by reverse-phase high-pressure liquid chromatography (HPLC) (Online Degaser, 717 Autosample, 660S Controller, 626 Pumps, 2996 Photo Diode Array; Waters Corporation, Milford, MA, USA) using a C18 column (5 μm, 250 × 3 mm, VYDAC 218 TP53; Grace-Davison, Deerfield, Illinois, USA). Twenty microliters was injected and elution was performed with a constant flow rate of 0.6 mL min^{-1} using a general program, allowing the simultaneous measurement of all three families of lipopeptides (Table S1, Supporting Information). Compounds were identified based on their retention times compared with commercial standards (98% purity, Lipofabrik, Villeneuve d'Ascq, France). HPLC peak areas were also used to quantify the three families of lipopeptides produced by strain 32a on the basis of values obtained for pure products.

B. velezensis 32a growth and lipopeptide production on different carbon sources

The effect of various carbon sources, typically found in tomato exudates, on growth and lipopeptide production by strain 32a was evaluated. Each substrate (glucose, fructose, maltose, ribose, citrate, succinate, malate, fumarate) was tested by adding a concentrated solution to a minimal medium composed of $(NH_4)_2SO_4$ 2 g L^{-1}, yeast extract 1 g L^{-1}, K_2HPO_4 1 g L^{-1}, KCl 0.5 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 0.5 g L^{-1}, $CuSO_4$ 1.6 mg L^{-1}, $Fe_2(SO_4)_3$ 1.2 mg L^{-1}, and $MnSO_4$ 0.4 mg L^{-1}. In all cases, pH was adjusted to 7 and the (C/N) ratio was (8:1). Cultures were inoculated with *B. velezensis* 32a or a combination of both *B. velezensis* 32a and *A. tumefaciens* C58 at a final concentration of 1×10^7 CFU mL^{-1}. They were incubated at 25 °C with agitation at 160 rpm. Bacterial growth in the presence of the different substrates was monitored 72 h after inoculation by plate count on LB medium. The supernatants obtained after centrifugation were filtered through a 0.22 μm membrane and processed for lipopeptide purification and quantification as described above. Each experiment was repeated three times.

Statistical analysis

All experimental results are expressed as mean with standard deviation (mean ± SD). The data were subjected to one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS V.11; SPSS Inc., Chicago, IL, USA). The mean values among the treatments were compared using the Duncan's multiple range test at the 5% level of significance ($p = 0.05$).

Heat map for growth (10^7 CFU mL^{-1}) and surfactin productivity (μg 10^{-8} CFU) by *B. velezensis* 32a was performed using Graph Pad Prism software (version 7.00, La Jolla, CA, USA).

Results

Effect of tomato root exudates on bacterial growth and lipopeptide production

The ability of *B. velezensis* 32a to grow and produce lipopeptides under the nutritional status imposed by tomato plants was evaluated. As shown in Table 1, the naturally produced exudates are conductive to the synthesis of lipopeptides by strain 32a. Surfactin, iturin, and fengycin families were produced in significant amounts under these conditions. Interestingly, the presence of *A. tumefaciens* C58 positively impacted the growth of the *Bacillus* strain. An increase in biomass density and much higher lipopeptide concentrations were generally measured upon confrontation with the pathogen (Table 1). These data confirm that exudate components are suitable for growth and lipopeptide synthesis by strain 32a.

Besides natural exudates, a recomposed tomato exudate medium containing organic acids (72%), sugars (23%), and amino acids (5%) was used to cultivate strain 32a, as determined by Kamilova et al. (2006). In both treatments, increased amounts of biomass and lipopeptides were measured compared with natural exudates (Table 1). Moreover, the relative proportions of the secreted lipopeptide families were almost similar in the natural and the recomposed exudates.

Rhizosphere colonization by *B. velezensis* strain 32a

The influence of *A. tumefaciens* C58 on 32a root colonization was studied using tomato plants grown aseptically under hydroponic conditions. The *Bacillus* colonization level was measured in 32a inoculated plants and compared with those co-inoculated with *A. tumefaciens* C58. In both uninfected and *Agrobacterium*-infected tomato seedlings, *B. velezensis* 32a readily established in the rhizosphere and persisted at high levels for up to 30 days after inoculation (Fig. 1). The kinetic of growth in both cases showed an increase of the 32a cells in the first period of incubation followed by a slight to reach a stable level of approximately 6.9×10^7 CFU g^{-1} root fresh weight. However, the presence of *A. tumefaciens* C58 significantly enhanced the rhizosphere colonization level of the *Bacillus* strain until the 12th day of incubation. In this period, a 5-fold increase in the population of *B. velezensis* 32a was observed in the roots of *Agrobacterium*-infected seedlings compared with those of uninfected seedlings.

Lipopeptide production by *B. velezensis* strain 32a during interaction with tomato seedlings

The lipopeptide production *in planta* by *B. velezensis* 32a was evaluated in the absence and presence of the pathogen *A. tumefaciens* C58. In both uninfected and *Agrobacterium*-infected tomato seedlings grown under hydroponic conditions, surfactin was the sole lipopeptide measured in relevant amounts among the bacterial products present in the rhizosphere extracts. As shown in Fig. 2, the kinetic of surfactin production showed a continuous increase in the secretion during the 30 days of incubation. However, the quantities of surfactins in the tomato root environment were much lower when *B. velezensis* 32a was grown in the presence of the bacterial pathogen *A. tumefaciens* C58. In all sampling times,

Table 1 Lipopeptide biosynthesis in tomato root exudates upon confrontation of strain 32a with *A. tumefaciens* C58

		Biomass (CFU X 10^7 mL^{-1})	LPs (mg L^{-1})			LPs[b] (μg 10^{-8} CFU)
			Surfactin	Iturin	Fengycin	
NE	32a	1.4 ± 0.6	4.4 ± 2.0	1.1 ± 0.1	16.0 ± 3.5	153.5
	32a + C58	3.3 ± 1.1	5.5 ± 1.8	nd[a]	12.2 ± 4.8	53.6
RE	32a	15.0 ± 9.4	45.7 ± 13.0	28.0 ± 12.0	330.0 ± 105.0	269.1
	32a + C58	57.7 ± 16.2	89.0 ± 17.0	nd	532.0 ± 148.0	107.6

[a] nd, not detected

[b] Total of surfactin, iturin and fengycin families

NE natural exudates, RE recomposed exudates

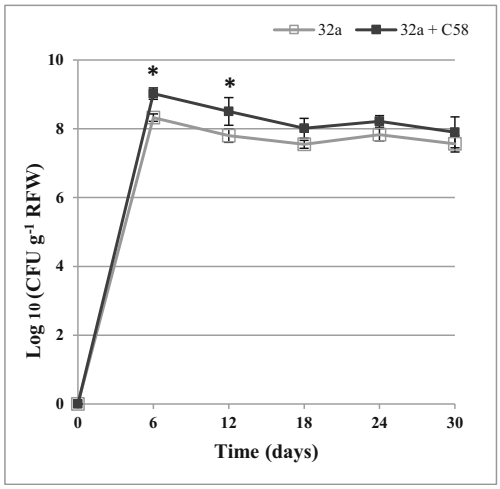

Fig. 1 Population density of *B. velezensis* 32a colonizing the roots of tomato plants, co-inoculated (black square) or not (white square) with *A. tumefaciens* C58. Error bars indicate ± SD of three replicates. Mean values significantly different ($p < 0.05$) according to Duncan test are marked by asterisks. RFW, root fresh weight

more than 2-fold higher amount of surfactins was detected in the rhizosphere extracts of uninfected seedlings than in those of *Agrobacterium*-infected seedlings.

Effect of different carbon sources on bacterial growth

To further understand the differential 32a colonization between uninfected and *Agrobacterium*-infected tomato seedlings, the effect of different sugars and organic acids typically found in the tomato root exudates on the growth of the *Bacillus* strain was evaluated. The carbon sources were tested individually in the absence and presence of the pathogen *A. tumefaciens* C58. The results are presented in Fig. 3.

In both cases, all the carbon sources supported the growth of *B. velezensis* 32a. In pure cultures, the highest biomass was

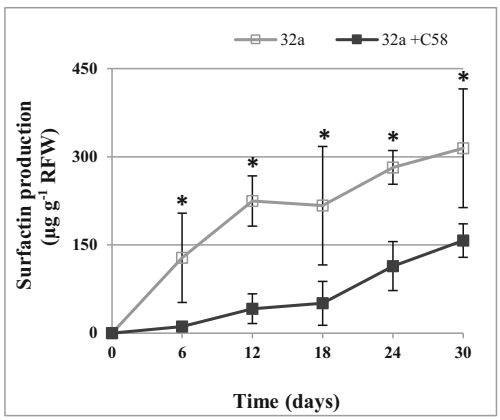

Fig. 2 Surfactin production by strain 32a colonizing roots of tomato plants, co-inoculated (black square) or not (white square) with *A. tumefaciens* C58. Error bars indicate ± SD of three replicates. Mean values significantly different ($p < 0.05$) according to Duncan test are marked by asterisks. RFW, root fresh weight

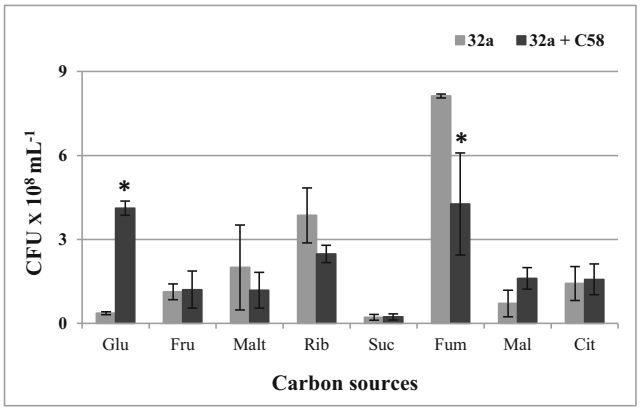

Fig. 3 *B. velezensis* 32a biomass production in the presence of various sugars and organic acids typically found in tomato exudates in presence (dark gray) or absence (light gray) of *A. tumefaciens* C58. Error bars indicate ± SD of three replicates. Mean values significantly different ($p < 0.05$) according to Duncan test are marked by asterisks. Glu, glucose; Fru, fructose; Malt, maltose; Rib, ribose; Suc, succinate; Fum, fumarate; Mal, malate; Cit, citrate

observed when fumaric acid was used as carbon source followed by ribose and maltose. In co-cultures, both fumaric acid and glucose were the best substrates. Comparison of 32a biomass obtained from pure and mixed cultures showed that the presence of the pathogen significantly impacted the growth of the *Bacillus* strain in some substrates. A positive effect was obtained with glucose, whereas fumaric acid and ribose influenced negatively the growth of *B. velezensis* 32a.

Effect of different carbon sources on lipopeptide production

The influence of the nutritional status imposed by tomato plant on lipopeptide production by strain 32a was further investigated to better explain the differential lipopeptide synthesis between uninfected and *Agrobacterium*-infected tomato seedlings. Sugars and organic acids of typical tomato exudates were individually tested, and lipopeptide production in pure and mixed cultures was determined and compared. The results are presented in Fig. 4.

A clear difference in lipopeptide production was observed between the carbon sources in both pure and mixed cultures. Surfactin secretion was more efficient in the presence of organic acids such as fumarate and malate than in the presence of sugars. By contrast, ribose was the best carbon source for iturin production. However, no measurable amount of iturin was observed when succinate was used. For fengycin, the highest production was obtained when fructose and malate were used as sugar and organic acid substrates, respectively.

Comparison of lipopeptide production of pure and mixed cultures showed that the presence of the pathogen significantly impacted the secretion of surfactin and fengycin, whereas no significant effect was observed for the iturin family (Fig. 4). As shown in Fig. 4a, lower amounts of surfactins were

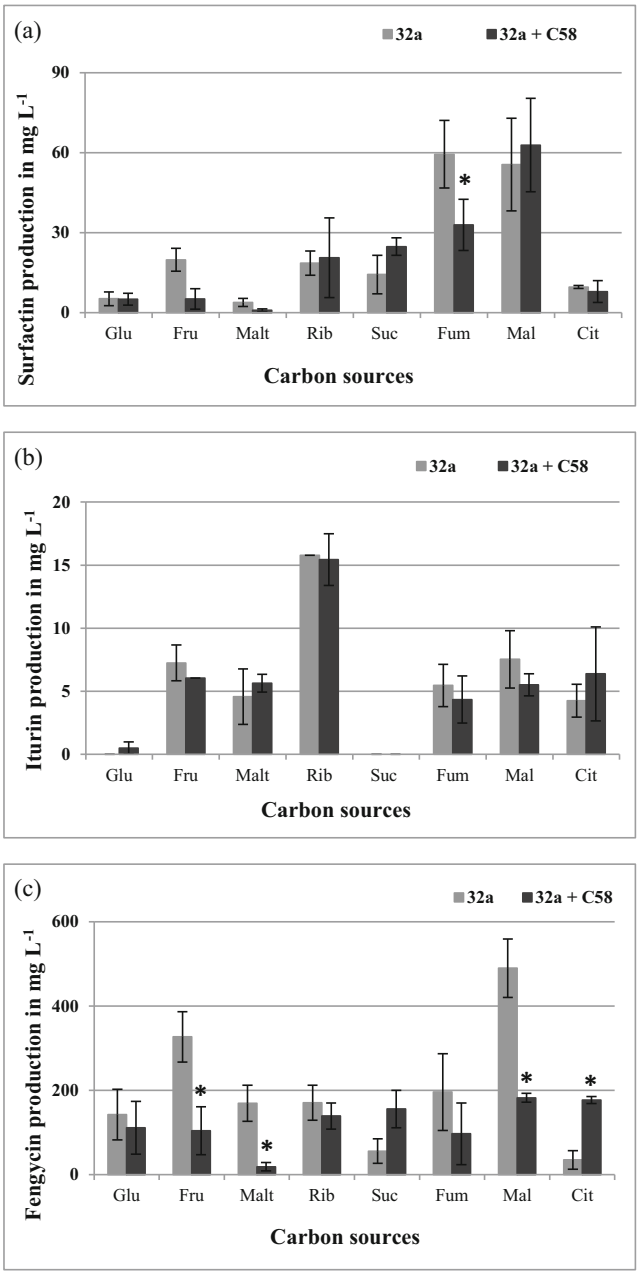

Fig. 4 Lipopeptide production by strain 32a; (a) surfactins; (b) iturins; (c) fengycins; in the presence of various sugars and organic acids typically found in tomato exudates in presence (dark gray) or absence (light gray) of *A. tumefaciens* C58. Error bars indicate ± SD of three replicates. Mean values significantly different ($p < 0.05$) according to Duncan test are marked by asterisks. Glu, glucose; Fru, fructose; Malt, maltose; Rib, ribose; Suc, succinate; Fum, fumarate; Mal, malate; Cit, citrate

detected in fumarate co-cultures compared with pure cultures. A negative impact on the production of fengycins was also observed, when fructose, maltose, and malate were used as a carbon sources (Fig. 4c). By contrast, the secretion of this lipopeptide was influenced positively with citrate as higher quantities of fengycins were measured on citrate co-cultures than in pure cultures.

Relationships between tomato root exudates, bacterial growth, and surfactin productivity

The effects of tomato root exudate composition on growth (10^7 CFU mL^{-1}) and surfactin productivity (μg 10^{-8} CFU) by strain 32a in the presence or absence of *A. tumefaciens* C58 were displayed in a gradient map (Fig. 5). The root exudate components were classified into two groups. The first dominant group consisted of glucose, fructose, malate, succinate, and citrate substrates having a positive or equal effect on 32a biomass between pure and mixed cultures with a negative effect on surfactin productivity in the presence of the pathogen. A second group consisted of maltose, ribose, and fumarate carbon sources having a negative effect on biomass in co-cultures compared with pure cultures with an equal or negative effect on surfactin productivity.

Discussion

An efficient production of lipopeptides is important for the biocontrol potential of the producing strains as well as for their ecological fitness in natural soil habitat. However, the specificity of the nutritional context imposed by the host plant and the complexity of the microbial communities sharing the ecosystem may affect the production of these biocontrol determinants under field conditions. In the present work, we evaluated

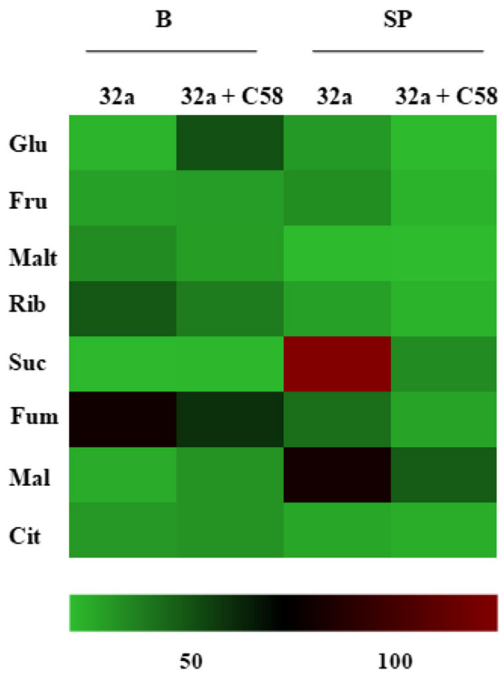

Fig. 5 Heat map showing the influence of tomato root exudates on biomass (10^7 CFU mL^{-1}) and surfactin productivity (μg 10^{-8} CFU) by strain 32a in presence or absence of *A. tumefaciens* C58. B, biomass; SP, surfactin productivity. The color scale depicts highest (red) via intermediate (black) to lowest (green) values for each variable

the impact of the bacterial pathogen *A. tumefaciens* C58 on growth and lipopeptide production by *B. velezensis* 32a under various nutritional contexts, representatives of natural conditions.

Like other members of *B. velezensis*, strain 32a is able to co-produce surfactin, iturin, and fengycin lipopeptides during its growth in rich medium (Ben Abdallah et al. 2015). However, the nutritional status of bacteria in synthetic media is qualitatively and quantitatively different from that in the rhizosphere. In this natural environment, nutriments provided by root exudates are limited and specific. Root-associated microorganisms are thus in nutrient-starved physiological state compared with rich culture media (Cawoy et al. 2015). Therefore, the ability of *B. velezensis* 32a to grow and produce lipopeptides in the presence of tomato root exudates as sole carbon source was further tested. Our data show that natural exudates are suitable for growth and lipopeptide synthesis by strain 32a in the absence and presence of the plant pathogenic bacterium *A. tumefaciens*. These results are also confirmed when the bacterium is cultured on tomato recomposed exudates, but with much higher levels of biomass and lipopeptide synthesis compared with natural exudates. This increase can be explained by variable concentrations of nutrients in the recomposed medium (Nihorimbere et al. 2009).

Results of in vivo experiments demonstrated that strain 32a is able to efficiently colonize the root surfaces of tomato plants grown under hydroponic conditions. The colonization process observed for the bacterial strain was similar to that commonly reported for rhizobacteria, with a first step of increasing in the population density followed by a decline to reach an almost stable level (Nihorimbere et al. 2009, 2012). Such steady-state phase probably corresponds to a resident phase where the population size is restricted by space and/or nutrient availability and is thus limited by plant growth and root exudation rate (Nihorimbere et al. 2009).

Confrontation with *A. tumefaciens* C58 resulted in a higher 32a population density in the roots of *Agrobacterium*-infected seedlings compared with those of uninfected seedlings. Such an increase in colonization by plant beneficial bacteria in the presence of soil-borne pathogens was also reported by other studies (Neveu et al. 2007; Jamali et al. 2009; DeCoste et al. 2010; Liu et al. 2014). This growth stimulation may be due to a change in the composition of root exudates by the plant at the perception of the pathogen to attract more beneficial bacteria (Kamilova et al. 2006; Rudrappa et al. 2008; Liu et al. 2017) or as a response of beneficial bacteria to signals received directly from the pathogen (DeCoste et al. 2010).

Natural conditions were found to be conductive to the synthesis of lipopeptides by 32a cells colonizing tomato roots. However, the lipopeptide signature of the bacterial strain is modulated upon growth in the tomato rhizosphere compared with in vitro conditions (planktonic cells grown in tomato root exudates). Indeed, surfactin was the sole lipopeptide clearly detected in the root environment of tomato seedlings. This is in agreement with other studies which revealed that surfactin is the main lipopeptide readily produced by cells evolving on plant roots (Nihorimbere et al. 2012; Debois et al. 2014; Zihalirwa Kulimushi et al. 2017). Some factors inherent to the development of *B. velezensis* in the rhizosphere may influence lipopeptide production such as change in the composition of tomato root exudates by the presence of the beneficial bacterium. It has been reported that beneficial microbes can modulate the plant root exudates (Kamilova et al. 2006; Etalo et al. 2018). This modulation strongly influenced the lipopeptide signature, as reported by Nihorimbere et al. (2012). The surfactin-enriched lipopeptide signature secreted *in planta* may be also due to the development of *Bacillus* cells as root-associated biofilm. Indeed, adhered cells were found to be very efficient in the production of surfactins than iturins and fengycins compared with cells living freely (Nihorimbere et al. 2009, 2012). The differential lipopeptide synthesis could likewise be explained by the slow growth rate of rhizobacterial cells colonizing roots, as shown by Nihorimbere et al. (2009).

The presence of *A. tumefaciens* C58 in the rhizosphere of tomato seedlings negatively impacted the production of surfactin by *B. velezensis* strain 32a. In fact, a much lower amount of surfactin was measured in the root environment of *Agrobacterium*-infected seedlings compared with the rhizosphere of uninfected seedlings. This decrease in surfactin production during the tripartite interaction may be due to the integration of the secreted lipopeptide in the membrane structure of *A. tumefaciens* C58 (Ongena and Jacques 2008), its enzymatic degradation by the soil-borne pathogen (Hoefler et al. 2012), or the production of a direct or indirect repressor of surfactin synthesis by *Agrobacterium* pathogen. A pH modification or a modulation in the composition of root exudates during the interaction between the plant and the two microbes is also possible (Kamilova et al. 2006; Liu et al. 2017).

To explain the observed changes occurring during the interaction between the two microbes *in planta*, the influence of the nutritional status imposed by the tomato plant on 32a growth and surfactin synthesis was investigated. Our results suggested that a modulation in root exudate composition is probably the cause of the differential 32a colonization and surfactin synthesis between uninfected and *Agrobacterium*-infected tomato seedlings. In fact, an increase in biomass and a decrease in surfactin productivity were observed in glucose and malate co-cultures compared with pure cultures. A reduced surfactin productivity was also measured in fructose, succinate, and citrate co-cultures while no significant effect on 32a growth was detected.

The *in planta* lipopeptide signature consisting on the sole production of surfactin in the tomato rhizosphere is of relevance in the context of biocontrol. In fact, the surfactin lipopeptide is involved in different mechanisms developed

by the producing bacteria for the biocontrol of phytopathogens (Ongena and Jacques 2008). Besides its antibacterial activity, it facilitates the colonization of plant roots by the producing strains and stimulates the host plant resistance potential (Raaijmakers et al. 2010; Zeriouh et al. 2011; Fan et al. 2017; Al-Ali et al. 2018). Direct antibiosis and stimulation of induced systemic resistance (ISR) are two important mechanisms used for controlling phytopathogens. However, unlike antibiosis, low amounts of surfactin (2–10 μM) are sufficient to induce ISR (Ongena et al. 2007; Jourdan et al. 2009). This latter mechanism was also suggested as more important than antibiosis in suppressing phytopathogens in the plant rhizosphere (Borriss 2015; Wu et al. 2015). Thereby, although the quantities of surfactin produced *in planta* were low and insufficient to provide a consistent antagonism against the bacterial pathogen *A. tumefaciens* under natural condition, these concentrations may be biologically relevant, considering its activity as an inducer of resistance in the host plant.

Conclusion

In this work, we studied for the first time the effect of *A. tumefaciens* C58 presence in the tomato rhizosphere on the level of root colonization and the amount of lipopeptide production by *B. velezensis* strain 32a. Our results show that interspecies interaction between the beneficial and the pathogenic microbes is one of the important factors that may affect the success of the biocontrol agent in the management of plant diseases by affecting its ecological fitness in the rhizosphere. This certainly represents an essential step for improving the efficacy of such biocontrol agents in the management of crown gall disease under the complex rhizosphere soil conditions.

More comprehensive investigations of interspecies chemical communication need to be undertaken for a better understanding of interactions among plants, *Agrobacterium* pathogens, and *Bacillus* beneficial microbes.

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