An Acad Bras Cienc (2022) 94(Suppl. 3): e20210860 DOI 10.1590/0001-3765202220210860
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CROP SCIENCE

Bacillus subtilis strain F62 against *Fusarium oxysporum* and promoting plant growth in the grapevine rootstock SO4

ALESSANDRA RUSSI, MARCUS ANDRÉ K. ALMANÇA & JOSÉLI SCHWAMBACH

Abstract: Fusarium wilt is a fungal disease that causes economic losses to viticulture, whose causal agent *Fusarium* sp. has been associated with the decline and death of young vines. This work had the objective of evaluating the antagonistic potential of *Bacillus subtilis* F62 against *F. oxysporum in vitro* and *in vivo*, as well as the growth promotion in the grapevine rootstock SO4. In the *in vitro* assay, the antagonism by diffusible and volatile compounds of *B. subtilis* F62 and the inhibition of conidial germination of four *Fusarium* sp. isolates were evaluated. In the *in vivo* assay, cuttings and micropropagated plants of SO4 were submitted to four treatments: control, Bac (*B. subtilis* F62 inoculation), Fus (*F. oxysporum* inoculation) and Bac + Fus. We observed that inhibition of mycelial growth occurred mainly by diffusible compounds. *B. subtilis* F62 had a positive effect on the growth promotion and in the biocontrol of *F. oxysporum*, reducing the frequency of pathogen re-isolation in cuttings (18.1%) and in micropropagated plants (52.4%). These results demonstrate the ability of *B. subtilis* F62 to upgrade plant development and assist in controlling of the Fusarium wilt in the grapevine rootstock SO4.

Key words: Biocontrol, Fusarium wilt, micropropagated plants, rootstock cuttings, *Vitis*.

INTRODUCTION

Brazilian vineyards have been affected by young vine decline and death recently. Many factors can contribute to this syndrome such as biotic agents involved in the grapevine trunk diseases, which are considered the main responsible for this decline. *Fusarium* spp. are soil-born phytopathogens that may cause Fusarium wilt, mainly in warm-climate vineyards (Dean et al. 2012, Compant et al. 2013) and might be related to this decline (Halleen et al. 2003, Garrido et al. 2004, Pintos et al. 2018, Akgül & Ahioğlu 2019).

F. oxysporum f. sp. *herbemontis* Tochetto was first detected in Brazil, in 1954, and up to now, has been detected in many regions of the country (Brum et al. 2012, Lerin et al. 2017). In Australia, *F. oxysporum* Schlecht caused root rot and reduced root biomass that resulted in weak and late shoots, as well as low vineyard productivity (Highet & Nair 1995). In South Africa, *Fusarium* sp. was isolated from vine shoots in nurseries showing symptoms of decline (Halleen et al. 2003). Damages to vineyards, caused by *Fusarium* sp., were also detected in Poland (Król 2006), in Egypt (Ziedan et al. 2011), in Japan (Cruz et al. 2014), in Iraq (Abdullah et al. 2015), in Greece (Markakis et al. 2017), in Italy (Reveglia et al. 2018), and in Turkey (Akgül & Ahioğlu 2019).

The genus *Fusarium* infects the vines through root wounds, causing plant wilt, root rot and longitudinal browning of the branches, yellowing and leaf detachment (Dean et al. 2012, Markakis et al. 2017). When the phytopathogen penetrates in the vascular system of the plants,

the xylem can be blocked leading to plant death by obstruction of water and nutrient transport (Brum et al. 2012, Eljounaidi et al. 2016).

The control of the disease is based on the use of resistant rootstocks and chemical pesticides (Costa et al. 2010), which can cause damage to the environment and human health, besides contributing to the selection of resistant strains of phytopathogens (Boubakri et al. 2015, Kejela et al. 2017). In addition, *Fusarium* sp. produces resistance structures, such as microsclerotia and chlamydospore, that can persist on the soil for long periods, making pathogen control difficult (Yadeta & Thomma 2013, Gramaje et al. 2018).

The mechanism of action of plant growth promoting rhizobacteria may involve increase in the plant development and productivity (Ahemad & Kibret 2014, Eljounaidi et al. 2016, Shafi et al. 2017), and the biocontrol of phytopathogens (Compant et al. 2013, Boubakri et al. 2015, Clemente et al. 2016). The biological control is based on the competition for space and nutrients, as well as the synthesis of antibiotics and the induction of host resistance (Tokpah et al. 2016).

Positive results in the biocontrol of *Fusarium* spp. on the vine were obtained using *Streptomyces* spp. (Ziedan et al. 2010) and *Pseudomonas fluorescens* (Ziedan & El-Mohamedy 2008, Svercel et al. 2010). Similarly, antagonistic effects of *Bacillus subtilis* were reported against *F. oxysporum* f. sp. *lentis* (El-Hassan & Gowen 2006), *F. circinatum* in *Pinus* (Soria et al. 2012) and *F. oxysporum* f. sp. *lycopersici* (Ramyabharathi et al. 2016). Thus, this work had as objective to evaluate the antagonistic activity of *B. subtilis* strain F62 in the *in vitro* and *in vivo* biocontrol of *F. oxysporum* and, simultaneously, evaluate the bacterial potential in the growth promotion of the rootstock SO4 (*Vitis berlandieri* x *V. riparia* Wall.), highly susceptible to this grapevine disease.

MATERIALS AND METHODS

Pathogen and bioagent isolates

Four isolates of *Fusarium* sp. (Table I) were obtained from vines showing symptoms of Fusarium wilt. They were morphologically characterized and stored in the fungal collections of the Laboratory of Phytopathology of the University of Caxias do Sul (three isolates), and the Federal Institute of Education Science and Technology of Rio Grande do Sul, Campus of Bento Gonçalves (one isolate). The pathogen isolate FusTD901, selected for *in vivo* experiments, was molecularly characterized as *F. oxysporum* by amplifying the ITS region. The antagonistic plant growth promoting bacterium was isolated from soil in Caxias do Sul, Brazil, and was preserved in the collection of the Laboratory of Phytopathology, University of Caxias do Sul, Brazil. For identification, it was submitted to the sequencing of 16S rRNA region, according to Sterky & Lundeberg (2000). The bioagent presented 100% similarity to a pre-existing sequence in NCBI of *B. subtilis*, at accession number NR 102783.2.

Initially, the bacteria were incubated in Potato Dextrose broth (PD) at 28 ºC for 48 h at 150 rpm in an orbital shaker. The cells were centrifuged at 3500 x g for 5 min at 23 ºC, the pellet was washed three times with sterilized water, resuspended in 0.85% NaCl solution, and the concentration was adjusted to 10^6 cfu mL^{-1}. The cell-free filtrate was obtained from the bacterial culture supernatant after 0.22 µm membrane filtration. The fungal conidia were

Table I. Isolates of *Fusarium* sp. used in the *in vitro* assays.

Isolates	Origin (city/country)	Institution
FusA9309	Caxias do Sul, Brazil	UCS
FusA4411	Bento Gonçalves, Brazil	UCS
FusTD901	Vacaria, Brazil	IFRS
FusA1215	Caxias do Sul, Brazil	UCS

obtained from 10-day old colonies incubated in Potato Dextrose Agar (PDA) at 28 ºC in 12 h light/12 h dark in a growth chamber and the spore concentration was adjusted to 10^5 conidia mL^{-1} with sterile water and Tween 80.

The experiments were conducted during 2017 and 2018, in the Laboratory of Grapevine Propagation, in Embrapa Grape and Wine, in Bento Gonçalves, Brazil.

In vitro antagonism

The bacterial antagonism against the mycelial growth of pathogens was evaluated in two different assays. In the antagonism by diffusible compounds (dual culture assay), a 6 mm diameter agar plug of each pathogen isolate was inoculated in the center of a plate containing PDA medium and after 24 hours, 25 µL of *B. subtilis* F62 suspension was applied at four points around fungal mycelium. The volatile compounds assay was evaluated using two plates containing PDA ovelaid and sealed with parafilm: on the upper plate was inoculated a 6 mm diameter mycelium plug and on the lower plate was spread 100 µL of *B. subtilis* F62 suspension. As a negative control, plates were inoculated only with pathogen isolates. Each treatment was replicated ten times and the plates were incubated in a growth chamber for 14 days at 25 °C in dark. The mycelial growth was daily measured using a digital caliper, and the data was converted into the mycelial growth rate index (MGRI) by using the formula: Σ [(d - dp)/N], where: d represents the mean colony diameter at the present day; dp represents mean colony diameter from the previous day; and N represents number of days after incubation. Furthermore, the mycelial growth inhibition (MGI) was determined in the 14^{th} day of the experiment according to [(dc – dt)/dc] × 100, where: dc and dt represent the mean colony diameters of control and treated groups, as described by Oliveira et al. (2016).

The conidial germination assay was carried out in flasks containing 5 mL of PD broth, in an orbital shaker at 130 rpm, 28 ºC for 24 h, in three different treatments: control = 10^5 conidia mL^{-1} of each pathogen isolate suspension; Bac + Fus = 10^5 cfu mL^{-1} of bacterial suspension + 10^5 conidia mL^{-1} of pathogen suspension; Fil + Fus = 1 mL of bacterial filtrate + 10^5 conidia mL^{-1} of pathogen suspension. The germination rate was evaluated by observing 100 conidia per replicate in an optical microscope, and each treatment was repeated three times. A conidium was considered germinated if the length of the germ tube exceeded half the length of the spore.

In vivo antagonism

Vegetative material of grapevine rootstock was collected on field in Embrapa Grape and Wine, in Bento Gonçalves, Brazil. Rootstock cuttings, measuring 30.0 cm length and 1.2 cm width, were hydrated for 24 h, submitted to hot water treatment, at 48-51 °C, during 30 min, according to Lerin et al. (2017) and incubated at 28 °C and 70% relative humidity for 15 days in a growth chamber. After five days of acclimation, they were transferred to plastic pots containing 250 mL autoclaved substrate (90% sphagnum peat and 10% vermiculite) with 5 g L^{-1} of gradual release fertilizer (5-6 months) and kept in a greenhouse.

In the *in vivo* assay, the pathogen isolate FusTD901 (*F. oxysporum*) was employed for presenting intermediary response in the antagonism with *B. subtilis* F62. The conidia suspension of the pathogen (5 x 10^5 conidia g^{-1} of substrate) was prepared according to Santos et al. (2016) and *B. subtilis* F62 was inoculated at the concentration of 10^4 CFU g^{-1} of substrate. Rootstock cuttings were submitted to four treatments, applied at three different

days (1, 7, and 14 days) after the beginning of the experiment: control = sterile water, Bac = *B. subtilis* F62 (1st and 14th days), Bac + Fus = *B. subtilis* F62 (1st and 14th days) + FusTD901 (7th day) and Fus = FusTD901 (7th day). The experiment was arranged in a completely randomized design with three replicates of twenty rootstock cuttings per treatment.

The experiment was kept in a greenhouse for 160 days, as described by Gramaje et al. (2016). After this period, the following parameters were assessed as described by Santos et al. (2016): length of the primary shoot (LPS), number of nodes in the primary shoot (NNPS), total number of shoots (TNS), total number of nodes (TNN), shoot dry weight (SDW), root dry weight (RDW) and frequency of pathogen re-isolation (RI). Dry weight was determined after drying plant material in forced ventilation at 60 ºC until constant weight. Pathogen re-isolation was carried out employing eight fragments from basal ends of the cuttings distributed in two Petri dishes.

The bacterial antagonism was also evaluated in micropropagated rootstocks. Shoots were collected from cuttings of SO4 submitted to hot water treatment as described above and, subsequently, in a water laminar flux cabinet, they were immersed in 70% ethanol for 1 min, followed by disinfestation with 1.0% sodium hypochlorite solution containing 0.02% Tween 20 for 20 min and rinsed three times with distilled and sterilized water. The propagules were inoculated in tubes containing 12 mL of half concentration MS medium (Murashige & Skoog 1962), supplemented with 3.0% (w/v) sucrose, 0.6% (w/v) agar, and 1 mg L^{-1} 6-benzylaminopurine. The medium was adjusted to pH 5.8 prior to autoclaving (121 ºC for 20 min). The explants were submitted to two subcultures, and the plantlets were rooted in same medium, supplemented with 1.5% (w/v) sucrose, 0.6% (w/v) agar, and 0.1 µg L^{-1} α-naphthaleneacetic acid. The cultures were maintained at 25 ± 2 °C, with a 16 h photoperiod (72 µmol m^{-2} s^{-1}) provided by fluorescent lamps in a growth chamber. *In vitro* rooted plantlets were washed before being transferred to plastic flasks containing 180 mL autoclaved substrate (90% sphagnum peat and 10% vermiculite) and acclimatized for 30 days at 23-28 °C, 70% relative humidity and 400 µmol m^{-2} s^{-1} of light intensity.

The *in vivo* assay with micropropagated rootstock was performed in triplicate with 30 replicates per treatment in a completely randomized design. The inoculum concentrations were the same described in rootstock cuttings. Plantlets were submitted to four treatments by drenching 4 mL of suspension onto substrate 1, 7, or 14 days before the beginning of the acclimatization: control = sterile water, Bac = *B. subtilis* F62 (1st and 14th days), Bac + Fus = *B. subtilis* F62 (1st and 14th days) + FusTD901 (7th day) and Fus = FusTD901 (7th day).

Plants were evaluated in three distinct periods: beginning of the assay; 30 days later: variation of leaf number (ΔLeaf1), variation of shoot length (ΔLength1); 160 days later: variation of leaf number (ΔLeaf2), variation of shoot length (ΔLength2), shoot dry weight (SDW), root dry weight (RDW) and frequency of pathogen re-isolation (RI).

Data analyses

All data were subjected to Kolmogorov-Smirnov test to check the normality. The *in vitro* antagonism of *B. subtilis* F62 was analyzed separately to volatile and diffusible compounds by ANOVA followed by t test and the isolates were compared among each other by ANOVA followed by Tukey test. The conidia germination assay was submitted to ANOVA and to Tukey post-hoc test. The interactions (isolates x treatments) for these assays were evaluated by Factorial ANOVA and

subsequently by Bonferroni test. In the *in vivo* assay, parametric data were analyzed by ANOVA followed by Tukey test and non-parametric data were analyzed by Kruskal-Wallis followed by Dunn-Bonferroni test. All the analyses were performed with SPSS 22.0 software (SPSS Inc. Chicago, IL), and the threshold for statistical significance was set at $p \leq 0.05$.

RESULTS

In vitro antagonism

The rhizobacterium *B. subtilis* F62 inhibited mycelial growth of all *Fusarium* sp. isolates in the antagonism by diffusible compounds. The mycelial growth rate index (MGRI) in dual culture assay showed reduction statistically significant concerning the control and it ranged from 1.47 (FusA4411) to 2.72 (FusA9309) (Table II). The mycelial growth inhibition (MGI), measured in the 14th day of the experiment, varied from 35.4% to 63.6% concerning the control (Figure 1 and Table II).

On the other hand, *Fusarium* sp. isolates submitted to antagonism by volatile compounds did not present any statistical difference in the MGRI (Figure 1 and Table II). Even though, sparse growth and morphological abnormalities in the fungal mycelium compared to the control was observed.

The conidia germination assay evaluated the effect of *B. subtilis* F62 suspension and cell-free filtrate on the spore germination. All the *Fusarium* sp. isolates presented conidia germinated after 24 hours from the beginning of the assay, even though FusA9303 had shown the lowest number of them (65.9). The treatments Bac +Fus and Filt + Fus inhibited conidia germination in all four isolates concerning the control. However, there was no statistically significant difference between the treatments Bac + Fus and Bac + Filt, for the isolates FusA9309, FusA1215 and FusTD901, while the isolate FusA4411 had a higher inhibition in the treatment Filt + Fus. Furthermore, among the pathogen isolates evaluated, FusA4411 and FusA1215 had a higher

Table II. Mycelial growth rate index (MGRI) of four isolates of *Fusarium* sp. alone and in the antagonism assay of *B. subtilis* F62 (Bac) by diffusible and volatile compounds, and the mycelial growth inhibition (MGI) concerning the control observed in 14th day of incubation.

Treatments	MGRI (mm/day) Diffusible	MGI (%) Diffusible	MGRI (mm/day) Volatile	MGI (%) Volatile
FusA9303	5.12 ± 0.12aA*		5.11 ± 0.13aAB	
FusA4411	5.28 ± 0.09aA*		5.27 ± 0.09aA	
FusTD901	5.16 ± 0.11aA*		5.18 ± 0.12aAB	
FusA1215	4.88 ± 0.22bA*		4.91 ± 0.24bB	
FusA9303 + Bac	2.72 ± 0.27aA	35.4	4.81 ± 0.20bB	0.0
FusA4411 + Bac	1.47 ± 0.23cB	63.6	5.33 ± 0.05aA	0.0
FusTD901 + Bac	1.81 ± 0.88bcB	56.4	4.73 ± 0.18bB	0.0
FusA1215 + Bac	2.15 ± 0.30abAB	49.8	4.71 ± 0.36bB	7.2

Statistical analyses were performed separately in the diffusible and volatile compounds assays.
Equal lowercase letters indicate no statistically significant difference among the isolates according to Kruskal-Wallis followed by Dunn-Bonferroni test ($p \leq 0.05$).
Equal uppercase letters indicate no statistically significant difference in the interaction treatments x isolates according to Factorial ANOVA followed by Bonferroni test ($p \leq 0.05$).
*** Asterisk indicates statistically significant difference between Fus (control) and Fus + Bac treatments (diffusible or volatile) according to ANOVA followed by t test ($p \leq 0.05$).**

spore germination concerning the isolates FusA9309 and FusTD901 in the treatment Bac + Fus. On the other hand, the isolates FusA9309, FusA4411, and FusTD901 had fewer spores germinated than the isolate FusA1215 submitted to Filt + Fus treatment (Figure 2).

In vivo antagonism

The application of *B. subtilis* F62 had a significant positive effect on the growth promotion of cuttings of SO4, increasing LPS, NNPS and TNN responses, while TNS, SDW and RDW responses did not differ statistically from the control (Table III).

Cuttings of SO4 infected with the pathogen FusTD901 showed similar responses to the control, whereas the treatment Bac + Fus promoted an increase in LPS, NNPS and SDW, minimizing the negative symptoms of the disease in the rootstock. Furthermore, the bacterium inoculation reduced the frequency of *F. oxysporum* re-isolation in SO4 cuttings from 75.8% in Fus treatment to 62.1% in Bac + Fus treatment, however, none of these results were statistically different (Table III).

In micropropagated rootstocks, the inoculation of *B. subtilis* F62 promoted a statistically significant increase of ΔLength1, ΔLeaf2, ΔLength2 and RDW, while the ΔLeaf1 and SDW responses did not differ statistically concerning the control (Table IV). On the other hand, the plants treated with the pathogen isolate FusTD901 (Fus treatment) presented lower ΔLeaf1, ΔLeaf2, ΔLength2 and SDW responses concerning the control and, rootstock susceptibility to Fusarium wilt. The Bac + Fus treatment had positive effect in all the responses evaluated (ΔLeaf1, ΔLeaf2, ΔLength1, ΔLength2, SDW and RDW) about the Fus treatment. Besides, there was a reduction in *F. oxysporum* re-isolation frequency from 59.9% in the Fus treatment to 28.5% in the Bac + Fus treatment (difference of 52.4%) (Table IV).

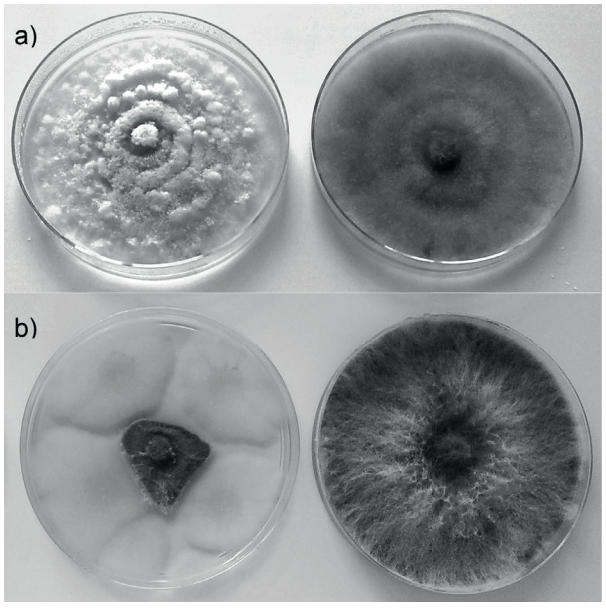

Figure 1. Mycelial growth of *F. oxysporum* isolate FusTD901, on the 14th day of growth, in the antagonism assay of *B. subtilis* F62 by volatile compounds (a) and by diffusible compounds/dual culture (b). The control is on the right side in both images.

DISCUSSION

In the current study, *B. subtilis* strain F62 demonstrated antagonistic activity by diffusible compounds against *Fusarium* sp. *in vitro* (varying from 35.4% to 63.6%). Similarly, Zhang et al. (2009) verified that 22 strains of *B. subtilis* inhibited the mycelial growth of *F. oxysporum* (ranging from 17 to 48%) and *F. graminearum* (ranging from 10 to 32%). Soria et al. (2012) studied the effect of four strains of *B. subtilis* and one strain of *Burkholderia* on the *in vitro* control of *F. circinatum* and found that bacterial metabolites reduced the growth rate by more than 50%. Antibiotics produced by *B. subtilis* EPCO16, in the dual culture assay, also inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici* in 44.44% (Ramyabharathi et al. 2016). So, it is possible to observe the efficiency of this bacterium

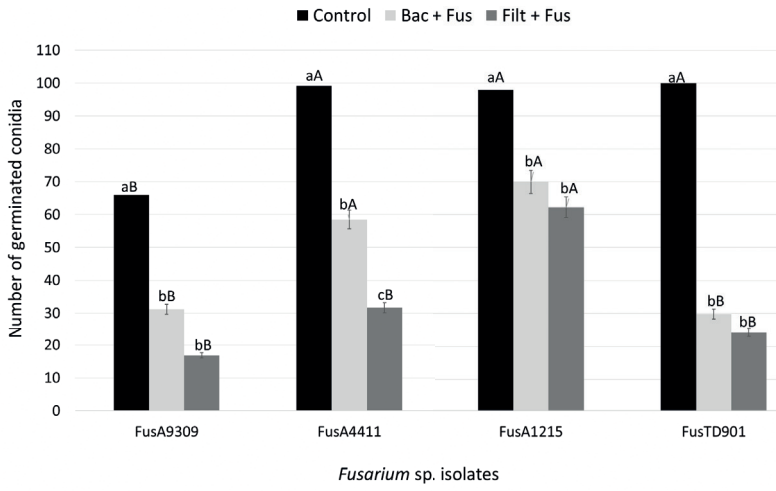

Figure 2. Number of germinated conidia of four *Fusarium* sp. isolates after 24 h of incubation. Values are the mean of three replicates and error bars indicate standard deviation. Equal lowercase letters indicate no statistically significant difference among the treatments (Control: *Fusarium* sp. conidia, Bac + Fus: *B. subtilis* suspension + *Fusarium* sp. conidia; Fil + Fus = *B. subtilis* cell-free filtrate + *Fusarium* sp. conidia) and equal uppercase letters indicate no difference among the isolates (FusA9309, FusA4411, FusA1215 and FusTD901), according to Factorial ANOVA followed by Bonferroni test ($p \geq 0.05$).

producing antifungal compounds and inhibiting pathogen growth under laboratory conditions.

Besides, other different bacteria had positive effect against *F. oxysporum*, corroborating our results. For example, Ziedan et al. (2010) observed that seven strains of *Streptomyces* spp. showed antagonistic activity against *F. oxysporum* in dual culture antagonism, especially *Streptomyces alni*, which promoted potent inhibition on fungal growth, causing hyphae malformation and lysis. In another study, *Pseudomonas* sp. strain pf4 inhibited the mycelial growth of *Colletotrichum gloeosporioides* in 41.67% and *F. oxysporum* in 48.14% (Manjunatha et al. 2012). Subsequently, Kejela et al. (2017) evaluated 40 isolates of *Pseudomonas* sp. and found that PT11 isolate showed 70% inhibition in the control of *C. gloeosporioides* and 72% in the control of *F. oxysporum* in dual culture assay.

The volatile compounds synthesized by *B. subtilis* F62 did not promote a reduction in the mycelial growth rate of *Fusarium* sp. Differently, Santos et al. (2016) observed that *B. subtilis* inhibited the mycelial growth of *Dactylonectria macrodidyma* from 29.5% to 69.1%. Wicaksono et al. (2017) also detected inhibition of 30% or more in the growth of Botryosphaeriaceae species using volatile compounds synthesized by three different strains of *Pseudomonas* sp. This behavior of *B. subtilis* F62 could be explained by the synthesis of different antifungal and volatile compounds that may cause inhibition of mycelial growth through damage and deformation in reproductive structures as hyphae, conidiophores, and conidia, supporting the abnormalities in fungal mycelium observed in our study. Similarly, Chaurasia et al. (2005) reported changes in the mycelium and hyphae morphology of *Alternaria alternata, Cladosporium oxysporum*, *F. oxysporum*, and *Pythium afertile* caused by volatile and diffusible compounds of *B. subtilis*.

Regarding the germination of *Fusarium* sp. conidia, both bacterial suspension and culture filtrate promoted inhibition, indicating that the presence of bacterial debris did not reduce the antifungal properties of the bioagent metabolites. Similarly, studies conducted by Sotoyama et al. (2016) demonstrated that both *B. amyloliquefaciens* IUMC7 suspension and bacterial culture filtrate inhibited the germination of conidia of *F. oxysporum* f. sp. *lycopersici*. Boubakri et al. (2015) also confirmed the inhibitory effect of *B. subtilis* strains Bs1 and Bs2 on the mycelial growth of *Botrytis cinerea* by filtered substances.

Table III. Morphophysiological responses in cuttings of grapevine rootstock SO4: length of the primary shoot (LPS), number of nodes in the primary shoot (NNPS), total number of nodes (TNN), total number of shoots (TNS), shoot dry weight (SDW), root dry weight (RDW) and frequency of *F. oxysporum* re-isolation (RI) in four treatments: control, *B. subtilis* F62 (Bac), *B. subtilis* F62 + FusTD901 (Bac + Fus) and FusTD901 (Fus).

Treatments	LPS**	NNPS*	TNN*	TNS*	SDW*	RDW*	RI**
	(cm)				(g)	(g)	(%)
Control	222.0 ± 13.1b	8.1 ± 0.3bc	8.8 ± 0.3b	1.2 ± 0.4a	2.2 ± 0.2a	2.1 ± 0.1ab	0.0 ± 0.0b
Bac	299.5 ± 7.7a	10.5 ± 0.2a	11.5 ± 0.3a	1.2 ± 0.4a	2.3 ± 0.1a	2.4 ± 0.1a	0.0 ± 0.0b
Bac + Fus	258.5 ± 8.7a	9.7 ± 0.2ab	10.0 ± 0.3ab	1.1 ± 0.3a	2.3 ± 0.2a	2.0 ± 0.1ab	62.1 ± 2.1a
Fus	185.0 ± 7.1b	7.5 ± 0.2c	8.2 ± 0.3b	1.1 ± 0.3a	1.1 ± 0.1b	1.6 ± 0.1b	75.8 ± 1.5a

*** Equal letters indicate no statistically significant difference according to ANOVA followed by Tukey test ($p \leq 0.05$).**
**** Equal letters indicate no statistically significant difference according to Kruskal-Wallis followed by Dunn-Bonferroni test ($p \leq 0.05$), for non-parametric responses.**

Several studies have described the potential of the filtrate of bacterial culture in the inhibition of conidial germination. Zhang et al. (2009) verified that metabolites produced by ten strains of *B. subtilis* promoted inhibition of macroconidia germination in *F. oxysporum* (varying from 20% to 48%) and in *F. graminearum* (from 14% to 32%) about the control. Benitez et al. (2010) observed that *B. amyloliquefaciens* LBM 5006 reduced the conidia germination and caused abnormal development of the germinative tube in *Aspergillus* spp., *Fusarium* spp., and *Bipolaris sorokiniana*. Similarly, Cao et al. (2012) reported that *B. subtilis* SQR 9 inhibited conidia germination of *F. oxysporum* f. sp. *cucumerinum,* and Gong et al. (2014) observed that the metabolite bacilomycin promoted 96.63% of inhibition on spore germination and 98.10% on sporulation of *Aspergillus flavus*.

In the *in vivo* assay using cuttings of SO4, *B. subtilis* F62 had a significant effect on growth promotion, mainly in the length of the primary shoot (LPS), number of nodes in the primary shoot (NNPS), and total number of nodes (TNN). Likewise, Santos et al. (2016) reported an increase in LPS and NNPS in grapevine cv. Merlot grafted onto one-year-old Paulsen 1103 treated with *B. subtilis*. However, the authors detected a reduction in the total number of nodes (TNN), total number of shoots (TNS), and dry mass of roots and shoots (RDW and SDW). On the other hand, Toffanin et al. (2016) evaluated the inoculation of *Azospirillum brasilense* Sp245 in the hydration stage and before grafting in cuttings of SO4 and verified an increase in the number of roots and total biomass. However, the cuttings treated in both stages did not present a statistically significant biomass increase. Different responses observed among these studies may be due to the tissue colonization, bacterial strain, and plant-bacteria interaction, also considering the grafting effect on plant development.

In the assay with micropropagated rootstocks of SO4, contrasting with the results observed in grapevine cuttings, *B. subtilis* F62 had a positive effect on growth, also in rootstocks previously infected with *Fusarium* sp. However, it is essential to consider that micropropagated plants did not have lignified tissues and significative amount of nutritional reserve, being more susceptible to bacterial activity on growth promotion than rootstock cuttings.

Similarly, Ziedan et al. (2010) verified that the inoculation of *Streptomyces* associated with the biofertilizer Rhizobacterin, which contains *Klebsiella planticola* BIM strain B-161, increased yield in grapevine cv. Superior.

Table IV. Morphophysiological responses in micropropagated rootstock SO4: variation of leaf number after 30 days (ΔLeaf1), variation of shoot length after 30 days (ΔLength1), variation of leaf number after 160 days (ΔLeaf2), variation of shoot length after 160 days (ΔLength2), shoot dry weight (SDW), root dry weight (RDW) and frequency *F. oxysporum* re-isolation (RI), in four different treatments: control, *B. subtilis* F62 (Bac), *B. subtilis* F62 + FusTD901 (Bac + Fus) and FusTD901 (Fus).

Treatments	ΔLeaf1**	ΔLength1**	ΔLeaf2**	ΔLength2*	SDW*	RDW**	RI**
		(cm)		(cm)	(g)	(g)	(%)
Control	2.3 ± 0.8a	10.9 ± 0.3c	12.7 ± 0.3b	592.9 ± 9.0b	3.6 ± 0.6a	1.7 ± 0.7c	0.0 ± 0.0c
Bac	2.6 ± 0.8a	23.4 ± 1.0a	16.6 ± 0.6a	680.7 ± 13.2a	3.8 ± 0.7a	3.5 ± 0.9a	0.0 ± 0.0c
Bac + Fus	2.3 ± 0.1a	16.0 ± 0.5b	13.7 ± 0.3b	617.7 ± 12.1b	3.5 ± 0.8a	2.7 ± 0.9b	28.5 ± 2.4b
Fus	1.3 ± 0.7b	11.0 ± 0.5c	11.2 ± 0.2c	511.6 ± 14.2c	2.9 ± 0.9b	1.3 ± 0.5c	59.9 ± 2.7a

*Equal letters indicate no statistically significant difference according to ANOVA followed by Tukey test ($p \leq 0.05$).
**Equal letters indicate no statistically significant difference according to Kruskal-Wallis followed by Dunn-Bonferroni test ($p \leq 0.05$).

Likewise, Hao et al. (2017) evaluated the effect of *Paenibacillus* sp. strain B2 in the growth promotion of micropropagated SO4 and biocontrol with co-inoculation with nematode *Xiphinema index*. They verified an increase in root biomass and growth promotion, also in plants co-inoculated with the nematode. Similar results were obtained employing growth-promoting bacteria in other cultures. El-Hassan & Gowen (2006) verified antagonistic effects of *B. subtilis* against *F. oxysporum* f. sp. *lentis*, in both seed and soil application, and dry matter increase in plants treated with the rhizobacteria. Ramyabharathi et al. (2016) observed high germination rates, shoot and root length treating tomato seeds with *B. subtilis* EPCO 16. Besides, Kejela et al. (2017) reported growth promotion, reduction in disease incidence, increase in germination rate, and increase in the activity of defense-related enzymes in coffee after treatment with *Pseudomonas* strain PT11.

A study carried out on cuttings of Sauvignon blanc submitted to treatments with *Pseudomonas* sp., *Burkholderia* sp. and *Serratia* sp. by drenching in the soil or inoculating in wounds on the trunk, there was the presence of *Pseudomonas* sp. I2R21 in branches, three centimeters above the wound, two months after inoculation (Wicaksono et al. 2017). On the other hand, the other endophytes could not colonize cuttings when inoculated in the soil, remaining for about four weeks after inoculation. According to Balmer et al. (2012), physical barriers such as the cell wall, antimicrobial toxins, and other defense mechanisms may hide the establishment and migration of endophytes. However, these bacteria were isolated from the mānuka plant (*Leptospermum scoparium*), and the vine was a heterologous host, which can suggest that different bacterial species or isolates have different host colonization abilities (Hardoim et al. 2008, Wicaksono et al. 2017). Different effects on growth promotion may be due to how bacterial colonization occurred since the root system colonization did not happen. This distinct colonization pattern is associated with the differentiated release of exudates by the plant, rhizobacteria growth rate, and bacterial host interactions (Compant et al. 2010). Moreover, composition of the exudates varies with genotype, stress conditions, stage of plant development, and even interaction with the natural soil microbiota (Haichar et al. 2008). These factors influence how bacteria colonize the roots and how migration to the internal tissues occurs later. After establishing

in plant tissues, the endophytic community presents a dynamic character, being influenced by physical-chemical characteristics of the soil, plant development phase, physiological and environmental conditions (Mercado-Blanco & Lugtenberg 2014). In addition, endophytic bacteria have a more intense relationship with the host than rhizobacteria (Rosenblueth & Martínez-Romero 2006).

Concerning the biocontrol of *F. oxysporum*, the different levels of fungal inhibition observed might be explained by phytopathogen virulence, bacterial colonization, mechanism of infection, and production of antifungal metabolites (Shafi et al. 2017). Besides, for the pathogen infection it is necessary the recognition of the host roots, the penetration of the hyphae, the degradation of physical barriers, the proliferation of hyphae, the adaptation to the defense responses of the plant, and the secretion of phytotoxins (Di Pietro et al. 2003). The infection changes the pattern of exudate release influencing bacterial colonization (Compant et al. 2010). The mechanism of action of endophytes involves the synthesis of lytic enzymes, antibiotics, and siderophores, which inhibit the development and infection of phytopathogens. Endophytes have different mechanisms of action against the phytopathogens in order to avoid resistance. Thus, if one way of biocontrol is not as effective, there will be distinct mechanisms to prevent or minimize infection (Eljounaidi et al. 2016).

In the present study, the activity of *B. subtilis* F62 against *F. oxysporum* was observed by bacterial inoculation in soil, both in cuttings and micropropagated plants (reduction in the incidence of 18.1% and 52.4%, respectively). In contrast, Baumgartner & Warnock (2006) found that soil application of *B. subtilis*, *B. lentimorbus*, *Comamonas testosteroni*, *Pseudomonas aeruginosa,* and *P. mendocina* on Cabernet Sauvignon vines grafted onto 110R (*V. berlandieri* × *V. rupestris*), did not control the root rot caused by *Armillaria mellea,* even though symptomatic plants treated with the bacteria demonstrated higher productivity and yield. Wicaksono et al. (2017) evaluated the effect of *Pseudomonas* sp. I2R21 and W1R33 on the biocontrol of the Botryosphaeriaceae species *Neofusicoccum luteum* and *N. parvum* on vine cuttings cv. Sauvignon blanc and found a reduction in the length of lesions caused by the pathogen from 32% to 52% concerning the control.

Biocontrol strategies play an important role against Fusarium wilt, whereas fungicides have failed to reduce the pathogen re-isolation and the disease symptoms (Bunbury-Blanchette et al. 2021, Yadav et al. 2021). Moreover, there is no efficient treatment to cure plants infected with vascular wilts, being recommended the remotion and destruction of these plants in the field (Yadeta & Thomma 2013, Gramaje et al. 2018).

The effectiveness of *Bacillus* genus in the biocontrol of *Fusarium* sp. has been reported in several cultures by reducing the phytopathogen incidence and disease severity. El-Hassan & Gowen (2006) verified antagonistic activity of *B. subtilis* with a reduction more significant than 70% in the incidence of *F. oxysporum* f. sp. *lentis* using the bioagent in the treatment of soil and seeds in lentil culture. Zhang et al. (2009) found that eight strains of *B. subtilis* reduced the severity of *F. oxysporum* and *F. graminearum* infection in soybean when inoculated in seeds or soil. There was a 43-63% reduction in disease severity in seed treatment, 18% increase in plant height, and 19% increase in root dry weight about control. In soil treatment, the disease severity reduction was higher, reaching 74%, plant length increased by up to 23%, and root dry weight by up to 24%. Ramyabharathi et al. (2016) verified that the inoculation of liquid

formulation *B. subtilis* strain EPCO16 in tomato seeds, soil inoculation, and leaf spray allowed a reduction in the incidence of *F. oxysporum* f. sp. *lycopersici* by up to 68.42%. Although the treatment with bacterial suspension did not eliminate the phytopathogen in these studies, *Bacillus* spp. can minimize the pathogen infection contributing to Fusarium wilt control, mainly when associated with other disease management strategies.

In summary, *B. subtilis* F62 demonstrated antagonistic potential *in vitro* against four *Fusarium* sp. isolates, reducing mycelial growth and conidia germination. *In vivo* assays have demonstrated the ability of this rhizobacterium to promote plant growth and reduce the percentage of *F. oxysporum* re-isolation. Further studies are need to confirm the bioagent potential in the control of Fusarium wilt in grapevine rootstocks, taking into account the responses observed in cuttings and micropropagated plants of rootstock SO4.

Acknowledgments

The funding for this research was provided by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

REFERENCES

ABDULLAH SK, AL-SAMARRAIE MQ & AL-ASSIE AH. 2015. Fungi associated with grapevine (*Vitis vinifera* L.) decline in middle of Iraq. Egypt Acad J Biol Sci 7: 53-59.

AHEMAD M & KIBRET M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26: 1-20.

AKGÜL DS & AHIOĞLU M. 2019. Fungal pathogens associated with young grapevine decline in the Southern Turkey vineyards. 42th World Congress of Vine and Wine. Geneva / Switzerland.

BALMER D, PLANCHAMP C & MAUCH-MANI B. 2012. On the move: induced resistance in monocots. J Exp Bot 64: 1249-1261.

BAUMGARTNER K & WARNOCK AE. 2006. A soil inoculant inhibits *Armillaria mellea in vitro* and improves productivity of grapevines with root disease. Plant Dis 90: 439-444.

BENITEZ LB, VELHO RV & LISBOA MP. 2010. Isolation and characterization of antifungal peptides produced by *Bacillus amyloliquefaciens* LBM5006. J Microbiol 48: 791-797.

BOUBAKRI H, HADJ-BRAHIM A, SCHMITTA C, SOUSTRE-GACOUGNOLLE I & MLIKI A. 2015. Biocontrol potential of chenodeoxycholic acid (CDCA) and endophytic *Bacillus subtilis* strains against the most destructive grapevine pathogens. New Zeal J Crop Hort 4: 261-274.

BRUM MCP, ARAÚJO WL, MAKI CS & AZEVEDO JL. 2012. Endophytic fungi from *Vitis labrusca* L. ('Niágara Rosada') and its potential for the biological control of *Fusarium oxysporum*. Genet Mol Res 11: 4187-4197.

BUNBURY-BLANCHETTE AL & WALKER AK. 2019. *Trichoderma* species show biocontrol potential in dual culture and greenhouse bioassays against Fusarium basal rot of onion. Biol Control 130: 127-135.

CAO Y, XU Z & LING N. 2012. Isolation and identification of lipopeptides produced by *B. subtilis* SQR 9 for suppressing Fusarium wilt of cucumber. Sci Hort 135: 32-39.

CHAURASIA B, PANDEY A, PALNI LMS, TRIVEDI P, KUMAR B & COLVIN N. 2005. Diffusible and volatile compounds produced by antagonistic *Bacillus subtilis* strain cause structural deformations in pathogenic fungi *in vitro*. Microbiol Res 160: 75-81.

CLEMENTE JM, CARDOSO CR, VIEIRA BSE, DA MATA FLOR I & COSTA RL. 2016. Use of *Bacillus* spp. as growth promoter in carrot crop. Afr J Agric Res 11: 3355-3359.

COMPANT S, CLEMENT C & SESSITSCH A. 2010. Plant growth promoting bacteria in the rhizo- and endosphere of plants. Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42: 669-678.

COMPANT S, BRADER G, MUZAMMIL S, SESSITSCH A, LEBRIHI A & MATHI F. 2013. Use of beneficial bacteria and their secondary metabolites to control grapevine pathogen diseases. Biol Control 58: 435-455.

COSTA MD, LOVATO PE & SETE PB. 2010. Micorrização e indução de quitinases e β-1,3-glucanases e resistência à fusariose em porta-enxerto de videira. Pesq Agropec Bras 45: 376-383.

CRUZ AF, PIRES MC, SOARES WRO, REZENDE DV & BLUM LEB. 2014. Soil-borne plant pathogens associated to decline

of grapevine grown in greenhouse. J Plant Physiol Pathol 2: 1-6.

DEAN R et al. 2012. The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13: 414-430.

DI PIETRO A, MADRID MP, CARACUEL Z, DELGADO-JARANA J & RONCERO MIG. 2003. *Fusarium oxysporum*, exploring the molecular arsenal of a vascular wilt fungus. Mol Plant Pathol 4: 315-325.

ELJOUNAIDI K, LEE SK & BAE H. 2016. Bacterial endophytes as potential biocontrol agents of vascular wilt diseases – Review and future prospects. Biol Control 103: 62-68.

EL-HASSAN SA & GOWEN SR. 2006. Formulation and delivery of the bacterial antagonist *Bacillus subtilis* for by *Fusarium oxysporum* f. sp. *lentis*. J Phytopathol 154: 148-155.

GARRIDO LR, SÔNEGO OR & GOMES VN. 2004. Fungos associados com o declínio e morte de videiras no estado do Rio Grande do Sul. Fitopatol Bras 29: 322-324.

GONG Q, ZHANG C & LU F. 2014. Identification of bacillomycin D from *Bacillus subtilis* fmbJ and its inhibition effects against *Aspergillus flavus*. Food Control 36: 8-14.

GRAMAJE D, ALANIZ S, ABAD-CAMPOS P, GARCÍA-JIMÉNEZ J & ARMENGOL J. 2016. Evaluation of grapevine rootstock against soilborne pathogens associated with trunk diseases. Acta Hortic 1136: 245-250.

GRAMAJE D, ÚRBEZ-TORRES JR & SOSNOWSKI MR. 2018. Managing grapevine trunk diseases with respect to etiology and epidemiology, current strategies and future prospects. Plant Dis 102: 12-39.

HAICHAR FZ, MAROL C, BERGE O, RANGEL-CASTRO JI, PROSSER JI, BALESDENT J, HEULIN T & ACHOUAK W. 2008. Plant host habitat and root exudates shape soil bacterial community structure. ISME J 2: 1221-1230.

HALLEEN F, CROUS PW & PETRINI O. 2003. Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. Australas Plant Pathol 32: 47-52.

HAO Z, VAN TUINEN D, WIPF D, FAYOLLE L, CHATAIGNIER O, LI X, CHEN B, GIANINAZZI S, GIANINAZZI-PEARSON V & ADRIAN M. 2017. Biocontrol of grapevine aerial and root pathogens by *Paenibacillus* sp. strain B2 and paenimyxin *in vitro* and *in planta*. Biol Control 109: 42-50.

HARDOIM PR, VAN OVERBEEK LS & VAN ELSAS JD. 2008. Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16: 463-471.

HIGHET AS & NAIR NG 1995. *Fusarium oxysporum* associated with grapevine decline in the Hunter Valley, NSW Australia. Aust J Grape Wine Res 1: 48-50.

KEJELA T, THAKKAR VR & PATEL RR. 2017. A novel strain of *Pseudomonas* inhibits *Colletotrichum gloeosporioides* and *Fusarium oxysporum* infections and promotes germination of coffee. Rhizosphere 4: 9-15.

KRÓL E. 2006. Fungi inhabiting decaying grapevine (*Vitis* spp.) cuttings. J Plant Prot Res 46: 353-358.

LERIN S, GROHS DS, ALMANÇA MAK, BOTTON M, MELLO-FARIAS P & FACHINELLO JC. 2017. Prediction model for phenology of grapevine cultivars with hot water treatment. Pesq Agropec Bras 52: 887-895.

MANJUNATHA H, NAIK MK, PATIL MB, LOKESHA R & VASUDEVAN SN. 2012. Isolation and characterization of native fluorescent pseudomonas and antagonistic activity against major plant pathogens. Karn J Agric Sci 25: 346-349.

MARKAKIS EA, KAVROULAKIS N, NTOUGIAS S, KOUBOURIS GC, SERGENTANI CK & LIGOXIGAKIS EK. 2017. Characterization offungi associated with wood decay of tree species and grapevine in Greece. Plant Dis 101: 1929-1940.

MERCADO-BLANCO J & LUGTENBERG B. 2014. Biotechnological applications of bacterial endophytes. Curr Biotechnol 3: 60-75.

MURASHIGE T & SKOOG FA. 1962. A revised medium for a rapid growth and bioassays with tobacco tissues cultures. Plant Physiol 15: 473-479.

OLIVEIRA TAS, BLUM LEB, DUARTE EAA, MOREIRA ZPM & LUZ EDMN. 2016. Variability of aggressiveness and virulence of *Phytophthora palmivora* influencing the severity of papaya fruit rot in postharvest in Bahia, Brazil. Científica 44: 185-195.

PINTOS C, REDONDO V, COSTAS D, AGUÍN O & MANSILLA P. 2018. Fungi associated with grapevine trunk diseases in nursery-produced *Vitis vinifera* plants. Phytopathol Mediterr 57: 407-424.

RAMYABHARATHI S, RAJENDRAN L, KARTHIKEYAN G & RAGUCHANDER T. 2016. Liquid formulation of endophytic *Bacillus* and its standardization for the management of *Fusarium* wilt in tomato. Bangladesh J Bot 45: 283-290.

REVEGLIA P, CINELLI T, CIMMINO A, MASI M & EVIDENTE A. 2018. The main phytotoxic metabolite produced by a strain of *Fusarium oxysporum* inducing grapevine plant declining in Italy. Nat Prod Res 32: 2398-2407.

ROSENBLUETH M & MARTÍNEZ-ROMERO E. 2006. Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interact 19: 827-837.

SANTOS RFD, HECKLER LI, LAZAROTTO M, GARRIDO LR, REGO C & BLUME E. 2016. *Trichoderma* spp. and *Bacillus subtilis* for control of *Dactylonectria macrodidyma* in grapevine. Phytopathol Mediterr 55: 293-300.

SHAFI J, TIAN H & JI M. 2017. *Bacillus* species as versatile weapons for plant pathogens, a review. Biotechnol Biotechnol Equip 1: 446-459.

SORIA S, ALONSO R & BETTUCCI L. 2012. Endophytic bacteria from *Pinus taeda* L. as biocontrol agents of *Fusarium circinatum* Nirenberg & O'Donnell. Chil J Agric Res 72: 281-284.

SOTOYAMA K, AKUTSU K & NAKAJIMA M. 2016. Biological control of Fusarium wilt by *Bacillus amyloliquefaciens* IUMC7 isolated from mushroom compost. J Gen Plant Pathol 82: 105-109.

STERKY F & LUNDEBERG J. 2000. Sequence analysis of genes and genomes. J Biotechnol 76: 1-31.

SVERCEL M, CHRISTEN D, MOËNNE-LOCCOZ Y, DUFFY B & DÉFAGO G. 2010. Distribution of *Pseudomonas* populations harboring phlD or hcnAB biocontrol genes is related to depth in vineyard soils. Soil Biol Biochem 42: 466-472.

TOFFANIN A, D'ONOFRIO C, CARROZZA GP & SCALABRELLI G. 2016. Use of beneficial bacteria *Azospirillum brasiliense* Sp245 on grapevine rootstocks grafted with 'Sangiovese'. Acta Hortic 1136: 177-184.

TOKPAH DP, LI H, WANG L, LIU X, MULBAH QS & LIU H. 2016. An assessment system for screening effective bacteria as biological control agents against *Magnaporthe grisea* on rice. Biol Control 103: 21-29.

WICAKSONO WA, JONES EE, MONK J & RIDGWAY HJ. 2017. Using bacterial endophytes from a New Zealand native medicinal plant for control of grapevine trunk diseases. Biol Control 114: 65-72.

YADAV K, DAMODARAN T, DUTT K, SINGH A, MUTHUKUMAR M, RAJAN S, GOPAL R & SHARMA PC. 2021. Effective biocontrol of banana fusarium wilt tropical race 4 by a bacillus rhizobacteria strain with antagonistic secondary metabolites. Rhizosphere 18: 100341.

YADETA KA & THOMMA BP. 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. Front Plant Sci 4: 1-12.

ZHANG JX, XUE AG & TAMBONG JT. 2009. Evaluation of seed and soil treatments with novel *Bacillus subtilis* strains for control of soybean root rot caused by *Fusarium oxysporum* and *F. graminearum*. Plant Dis 93: 1317-1323.

ZIEDAN EHE & EL-MOHAMEDY RSR. 2008. Application of *Pseudomonas fluorescens* for controlling root-rot disease of grapevine. Res J Biol Sci 4: 346-353.

ZIEDAN ESH, EMBABY ESM & FARRAG ES. 2011. First record of Fusarium vascular wilt on grapevine in Egypt. Arch Phytopathol Plant Prot 44: 1719-1727.

ZIEDAN ESH, FARRAG ES, EL-MOHAMEDY RS & ABD ALLA MA. 2010. *Streptomyces alni* as a biocontrol agent to root-rot of grapevine and increasing their efficiency by biofertilisers inocula. Arch Phytopathol Plant Prot 43: 634-646.

How to cite

RUSSI A, ALMANÇA MAK & SCHWAMBACH J. 2022. *Bacillus subtilis* strain F62 against *Fusarium oxysporum* and promoting plant growth in the grapevine rootstock SO4. An Acad Bras Cienc 94: e20210860. DOI 10.1590/0001-3765202220210860.

Manuscript received on June 15, 2021;
accepted for publication on January 25, 2022

ALESSANDRA RUSSI[1]
https://orcid.org/0000-0002-2487-252X

MARCUS ANDRÉ K. ALMANÇA[2]
https://orcid.org/0000-0002-3895-4390

JOSÉLI SCHWAMBACH[3]
https://orcid.org/0000-0001-6785-4164

[1]Embrapa Uva e Vinho, Laboratório da Propagação da Videira, Rua Livramento, 515, 95701-008 Bento Gonçalves, RS, Brazil

[2]Instituto Federal de Educação Ciência e Tecnologia do Rio Grande do Sul, Laboratório de Fitopatologia, Avenida Osvaldo Aranha, 540, 95700-206 Bento Gonçalves, RS, Brazil

[3]Universidade de Caxias do Sul, Instituto de Biotecnologia, Rua Francisco Getúlio Vargas, 1130, 95070-560 Caxias do Sul, RS, Brazil

Correspondence to: **Joséli Schwambach**
E-mail: jschwambach@ucs.br

Author contributions

Alessandra Russi: investigation, methodology, resources, software, validation, writing-original draft, writing-review and editing. Marcus André Kurtz Almança: investigation, methodology, supervision, writing-review and editing. Joséli Schwambach: funding acquisition, investigation, methodology, project administration, supervision, writing-review and editing.