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Biocontrol and plant-growth-promoting capacities of actinobacterial strains from the Algerian Sahara and characterisation of *Streptosporangium becharense* SG1 as a promising biocontrol agent

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ABSTRACT

Sixteen actinobacterial strains isolated from various ecological niches in the Algerian Sahara were screened for their biocontrol potential in root rot disease caused by *Fusarium culmorum* and their promotion of durum wheat growth. All actinobacteria were studied for *in vitro* antagonistic activity and plant-growth-promotion traits, for the production of cyanhydric acid, siderophores, chitinases and indole-3-acetic acid, and for the solubilisation of inorganic phosphate. Strongly antagonistic actinobacteria were selected for the biocontrol of *F. culmorum in vivo* and for the growth promotion of durum wheat plants in autoclaved and non-autoclaved soils. The *Streptosporangium becharense* strain SG1 exhibited remarkable positive results in all trials. Compared to untreated wheat seeds, the root rot severity index was decreased significantly ($P < 0.05$) by all seed bacterization treatments. However, the highest protective effect was obtained by the strain SG1, which reduced the disease severity index from 77.8% to 16%, whereas it was only reduced to 24.2% by chemical seed treatment with Dividend®. Moreover, strain SG1 led to significant increases in the shoot length, root length and dry weight of plants, thus opening up interesting perspectives for possible exploration in crop enhancement.

KEYWORDS

Actinobacteria; *Streptosporangium becharense* strain SG1; biocontrol; *Fusarium culmorum*; plant-growth-promotion; durum wheat

Introduction

Chemical products are commonly used as pesticides or fertilisers to improve crop production. However, the abusive use of agrochemical compounds often causes problems such as contamination of the soil, high toxicity on native microbial communities, pesticide resistance and other adverse effects on the environment (Huang, Zhang, Yong, Yang, & Shen, 2012) as well as the potential harmful effects on human health (Ippolito & Nigro, 2000).

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Root rot and damping-off of seedlings is a common disease caused by *Fusarium* species in a variety of crop cereals, such as corn, rice, barley and wheat. In Algeria, *Fusarium culmorum* is considered to be a serious problem for the cereal crops, as it causes significant losses, particularly at the seedling stage (Yekkour et al., 2012). Various fungicides are frequently used to manage the *Fusarium* diseases and to prevent crop losses. Nevertheless, the majority of them are not ideally effective to eradicate these phytopathogenic fungi (Huang et al., 2012). Because of these preoccupations, there is a rising demand for biocontrol methods that are applicable in sustainable agriculture in order to protect the environment by reducing chemical pesticide use (Shimizu, 2011).

Actinobacteria are considered as potential biocontrol agents of plant diseases. De-Oliveira, Da Silva, and Van Der Sand (2010) reported that *Streptomyces* sp. strain R18 could colonise the plant rhizosphere soil and produce active molecules such as antibiotics, hydrolytic enzymes, hydrogen cyanide, and siderophores. El-Tarabily, Nassar, and Sivasithamparam (2008), reported the efficacy of *Micromonospora endolithica* in phosphate solubilisation, and Cao, Qiu, You, Tan, and Zhou (2005) reported that the endophytic *Streptomyces* sp. strain S 96, isolated from tissues of banana roots, produced siderophores and was effective to protect banana plantlets against *F. oxysporum* f. sp. *cubense*.

Actinobacteria of the genus *Streptomyces* are also known to develop symbiotic associations with crop plants, colonising their internal tissues without causing disease symptoms and producing phytohormones such as gibberellic acid and indole-3-acetic acid (IAA) (Goudjal et al., 2013). In addition, several researchers have reported the potential of plant-associated actinobacteria as agents to manage various soil-borne phytopathogenic fungi and/or to stimulate plant growth. Costa, Zucchi, and De Melo (2013) reported the efficacy of endophytic *Streptomyces* strains to reduce the incidence of damping-off caused by *Pythium aphanidermatum* in cucumber (*Cucumis sativa* L.) El-Tarabily, Hardy, and Sivasithamparam (2010) highlighted the growth promotion effect of endophytic *Streptomyces spiralis*, *Actinoplanes campanulatus* and *Micromonospora chalcea*, individually and in combination, on cucumber seedlings.

In this context, we aimed to evaluate the potential of actinobacterial strains from sandy soils or native plants that had successfully adapted to the harsh cultural conditions of the Algerian Sahara, as biocontrol agents of *F. culmorum* root rot disease *in vivo* and for promoting the growth of durum wheat plants.

Materials and methods

Actinobacterial strains

Sixteen rhizospheric or endophytic actinobacteria (Table 1), isolated by our research team in the *Laboratory of Biology of Microbial Systems (LBSM), ENS – Kouba, Algiers, Algeria*, were tested for their *in vivo* biocontrol efficacy on *Fusarium culmorum* root rot disease and in the growth promotion of wheat plants. Actinobacteria were selected based on their effectiveness in the biocontrol of soil-borne phytopathogenic fungi such as *Rhizoctonia solani* (Goudjal et al., 2014) and *F. oxysporum* f. sp. *radicis-lycopersici* (Zamoum et al., 2015), on their promotion effect on cropped plants (Goudjal et al., 2013) and on the fact that they were assigned as novel species of actinobacteria (Chaabane Chaouch et al., 2016a, 2016b; Lahoum et al., 2016).

Table 1. Ecological niches of actinobacterial strains and their antagonistic activities towards soil-borne phytopathogenic fungi.

Isolate		Ecological niche (Rhizospheric soil or host plant)	Antagonistic activity (zone of inhibition in mm)[a]				
			Fusarium culmorum (LF18)	*F. graminearum* (LF21)	*F. oxysporum* f. sp. *radicis-lycopersici* (LF30)	*Rhizoctonia solani* (LAG3)	*Bipolaris sorokiniana* (LB12)
Rhizospheric isolates	*Saccharothrix longispora* strain MB29*	Saharan soil from Ghardaïa	27 ± 0.8	5 ± 0.49	16 ± 0.7	15 ± 0.2	10 ± 0.7
	Actinomadura algeriensis strain ACD1 (Lahoum et al., 2016)	Saharan soil from Hoggar	10 ± 0.14	7 ± 0.6	00	00	7 ± 0.2
	Streptomyces sp. strain ZS1*	Saharan soil from Laghouat	13 ± 0.5	10 ± 0.25	10 ± 0.7	12 ± 0.6	9 ± 0.79
	Streptosporangium becharense strain SG1 (Chaabane Chaouch et al., 2016b)	Saharan soil from Béchar	28 ± 0.1	22 ± 0.5	28 ± 0.1	27 ± 0.2	23 ± 0.7
	Streptosporangium sp. strain SG10*	Saharan soil from Béchar	18 ± 0.3	1 ± 0.5	/	/	00
	Streptosporangium saharense strain SG20 (Chaabane Chaouch et al., 2016a)	Saharan soil from Ghardaïa	12 ± 0.1	9 ± 0.3	00	00	15 ± 0.4
Endophytic isolates	*Streptomyces* sp. strain APU4*	*Aristida pungens*	00	00	00	40 ± 0.2	00
	Streptomyces cyaneofuscatus strain ARR2 (Goudjal et al., 2014)	*Astragalus armatus*	00	00	00	40 ± 0.2	00
	Streptomyces mutabilis strain CAR2 (Goudjal et al., 2014)	*Cleome arabica*	25 ± 0.2	8 ± 0.1	58 ± 0.3	20 ± 0.1	10 ± 0.1
	Streptomyces sp. strain DNT4*	*Phoenix dactylifera*	24 ± 0.3	10 ± 0.2	00	20 ± 0.3	00
	Streptomyces sp. strain MLA2*	*Medicago laciniata*	17 ± 0.8	21 ± 0.2	25 ± 0.1	20 ± 0.1	16 ± 01
	Streptomyces rochei strain PTU2 (Goudjal et al., 2013)	*Panicum turgidum*	16 ± 0.2	26 ± 0.1	30 ± 0.3	59 ± 0.1	15 ± 0.2
	Streptomyces sp. Strain SNl6*	*Solanum nigrum*	00	10 ± 0.4	00	15 ± 0.1	00
	Streptomyces sp. strain TLE4*	*Terfezia leonis*	20 ± 0.7	27 ± 0.8	18 ± 1.7	24 ± 0.9	00
	Streptomyces neopeptinius strain TLE8 (Goudjal, Zamoum, & Meklat, et al., 2016)	*Terfezia leonis*	23 ± 0.7	7 ± 0.12	18 ± 0.2	12 ± 0.9	00
	Streptomyces caeruleatus strain ZLT2 (Zamoum et al., 2015)	*Ziziphus lotus*	27 ± 0.3	25 ± 0.2	60 ± 0.4	20 ± 0.1	19 ± 0.5

[a] = Standard deviation from three replicates.
/ =Test not performed.
*Strain from the actinobacterial collection of the *Laboratory of Biology of Microbial Systems (LBSM), ENS – Kouba, Algiers, Algeria.*

Antagonistic activity of endophytic actinobacteria

The streak method was used to estimate the antagonistic activities of actinobacteria against five soil-borne pathogenic fungi (*Fusarium culmorum* (LF18), *F. graminearum* (LF21), *F. oxysporum* f sp. *radicis-lycopersici* (LF30), *Rhizoctonia solani* (LAG3), and *Bipolaris sorokiniana* (LB12)) from the microbial collection of our laboratory. The actinobacterial strains were cultivated separately in straight lines on International *Streptomyces* Project (ISP) 2 medium (BD-DIOFO^{TM}) plates, which were incubated for 8 days at 30°C. After that, target fungi were seeded in lines perpendicular to those of actinobacteria cultivation. After incubation at 25°C for 5 days, the distance of inhibition between target fungus and actinobacteria colony margins was measured (Toumatia et al., 2015).

Determination of biocontrol and plant-growth-promotion traits

Hydrogen cyanide (HCN) production

Actinobacteria were grown in Bennett agar (HiMedia) amended with 4.4 g l^{-1} of glycine to study their ability to produce HCN. A Whatman filter paper grade 1 (125 mm diameter; medium porosity) was soaked with 0.5% picric acid in 2% sodium carbonate for one minute and attached under the Petri dish lid. The plates were then sealed with parafilm and incubated for 7 days at 30°C. The filter paper took on an orange colour when HCN was produced (Passari et al., 2015).

Siderophore production

The method described by Sadeghi et al. (2012) was used to evaluate the production of siderophores. Six millimetre disks from actinobacteria cultures were placed on chrome azurol S (Fluka) plates and incubated at 30°C for 7 days. The apparition of orange haloes around colonies was indicative of siderophore production.

Chitinolytic activity

The actinobacteria were spot inoculated on colloidal chitin agar medium (Gonzalez-Franco, Deobald, Spivak, & Crawford, 2003) to test for chitinase production. Cultures were incubated at 30°C for 5 days. Chitinolytic activity was estimated by measuring the diameter of the hydrolytic halo surrounding the actinobacterial colonies (Zamoum, Goudjal, Sabaou, Mathieu, & Zitouni, 2017).

Indole-3-acetic acid production (IAA)

To assess IAA synthesis, actinobacterial strains were inoculated into Erlenmeyer flasks containing 50 ml of yeast extract-tryptone (YT) broth (HiMedia), supplemented with 5 mg ml^{-1} of L-tryptophan, and kept in an incubated shaker (200 rpm, 30°C, 5 days). The flasks containing the culture broth were then centrifuged at 4000 rpm for 30 min. An equimolar concentration of Salkowski reagent (1 ml 0.5 M $FeCl_3$ dissolved in 50 ml 35% $HClO_4$) was added to 2 ml of supernatant. The mixture was incubated in the dark for 30 min and the appearance of pink colour indicated the production of indole compounds. The production of IAA was then confirmed by thin layer chromatography (TLC) as used by Ahmad, Ahmad, and Khan (2005). Ethyl acetate fractions (10–20) were spotted on TLC plates (silica gel GF254, thickness 0.25 mm, Merck, Germany)

and developed in ethyl acetate: chloroform: formic acid (55:35:10, by vol.). Spots with R_f values identical to authentic IAA were identified under UV light after the plates had been revealed with Ehmann's reagent. The absorbance was measured in a spectrophotometer at 530 nm and the IAA concentration was calculated using a pure IAA standard curve (Goudjal et al., 2013).

Phosphate solubilisation

The assays were conducted in 500 ml Erlenmeyer flasks containing 100 ml of Pikovskaya broth (HiMedia) amended with 5 g l^{-1} of $Ca_3(PO_4)_2$, $AlPO_4$ or $FePO_4$ as insoluble phosphate sources. Flasks were inoculated with 1 ml aliquot ($\approx 10^6$ CFU ml^{-1}) of the actinobacterial spore suspensions and incubated on a rotary shaker (200 rpm, 30°C) for 7 days. The cultures were then centrifuged for 10 min at 10000 rpm and the culture supernatants were collected and used to determine the amount of dissolved phosphorus using the molybdenum blue colorimetric method (Liu et al., 2014).

In vivo biocontrol of Fusarium culmorum

The efficacy of the strong antagonistic actinobacteria in the *in vivo* biocontrol of *F. culmorum* (LF18) and their ability to promote the growth of durum wheat (cv. vitron) seedlings were investigated in an infested soil sampled from a cereal field in the Algerian Sahara (33° 62' N, 2° 91' E). Trials were performed both in autoclaved (120°C for 20 min) and non-autoclaved soils.

Surface-sterilization of seeds was performed by sequential dipping in 70% (v/v) ethanol solution for 3 min and 0.9% (v/v) NaClO solution for 4 min, followed by washing three times in sterile distilled water. The surface-sterilized seeds were then separately bacterized by dipping in the actinobacterial spore suspension ($\approx 10^6$ CFU ml^{-1}) for 30 min. They were dried under a laminar flow hood before being sown the same day. Actinobacteria spores on the bacterized seeds were counted by the plate dilution method. Decimal dilutions of actinobacterial spore suspensions were prepared using physiological saline before isolation on ISP2 medium. Actinobacterial colonies were counted after incubation at 30°C for 7 days. Bacterized seeds yielded $\approx 4 \times 10^6$ CFU g^{-1} seeds.

Autoclaved and non-autoclaved soils were infested with the *F. culmorum* spore suspension ($\approx 10^3$ CFU ml^{-1}). For this purpose, plastic pots (10 cm in diameter × 12 cm high) filled with soil were irrigated with 100 ml of the *F. culmorum* spore suspension. The density of *F. culmorum* in the infested soil was evaluated using the plate count method on PDA plates as described above. The *Fusarium* density was approximately 1.11×10^4 CFU g^{-1}.

Four treatments were conducted in the biocontrol assay: (1) untreated seeds were sown in non-infested pots (negative control); (2) untreated seeds were sown in infested soils to highlight the virulence of *F. culmorum* (LF18) (positive control); (3) bacterized seeds were sown in pots with infested soil to evaluate the biocontrol potential of each antagonistic actinobacteria strain; (4) surface-sterilized seeds were treated with a commercial chemical fungicide [Dividend® 030 FS (Difenoconazole)] by dipping in the fungicide solution for 3 min and drying under a laminar flow hood for 2 h, before being cultivated in infested soils.

Five seeds were sown per pot with 10 replicates for each treatment. In vivo biocontrol trials were conducted twice to ensure reproducibility. Pots were then placed in a fully randomised complete block design in a greenhouse (24–28°C, 14 h light/10 h dark). Cultures were irrigated daily with tap water (10 ml per pot) for 6 weeks.

The *F. culmorum* root rot symptoms were evaluated using the following scale (Dhanasekaran et al., 2005): 0 = no symptom, 1 = 0–25% of root browning, 2 = 26–50% of root browning, 3 = 51–75% of root browning, 4 = 76–100% of root browning and 5 = plant death. The Digimizer image analysis software was used to evaluate the area of root browning. For each seed treatment, the disease severity index (DSI) was calculated using the following formula:

$$DSI(\%) = \left[\sum (R \times N)\right] \times 100 / (H \times T)$$

R = the disease rating, N = number of plants with this rating, H = highest rating category, T = total number of plants counted for each treatment.

The effect of each seed treatment on the growth of wheat plants was also evaluated by measuring the shoot and root lengths, again using the Digimizer image analysis software. The healthy seedlings were then dried at 80°C until their weight became constant.

Statistical analysis

Three replications were performed for each experiment (10 replicates for *in vivo* trials) and values represent the mean ± standard deviation. Data were subjected to an analysis of variance (ANOVA). When the F-statistic was significant, Tukey's post hoc test ($P = 0.05$) was used to separate means. A principal component analysis (PCA) was carried out using the *XLStat* software to highlight the biocontrol potential and plant-growth-promoting activity of each actinobacterial strain.

Results

Antagonistic activities

Of the 16 actinobacterial strains tested, seven (43.8%) showed positive antagonistic activities against all targeted fungi. Ten strains (62.5%) showed antagonistic effects against at least three of the five soil-borne phytopathogenic fungi tested (Table 1), the most striking antagonistic activity being against *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Rhizoctonia solani*. Mycelial growth of *F. culmorum* was inhibited by only four (25%) actinobacterial strains. Strong antifungal activities (inhibition zone >20 mm) were noted in seven strains (*Streptomyces neopeptinius* TLE8, *Streptomyces* sp. TLE4, *Streptomyces mutabilis* CAR2, *Streptomyces* sp. DNT4, *Saccharothrix longispora* MB29, *Streptosporangium becharense* SG1 and *Streptomyces caeruleatus* ZLT2),the largest inhibition zone being observed for *S. becharense* SG1.

Hydrogen cyanide, siderophore production and chitinolytic activity

Results for HCN and siderophore production, and chitinolytic activity shown by the strongly antagonistic actinobacterial strains (*Streptomyces neopeptinius* TLE8,

Streptomyces sp. TLE4, *Streptomyces mutabilis* CAR2, *Streptomyces* sp. DNT4, *Saccharothrix longispora* MB29, *Streptosporangium becharense* SG1 and *Streptomyces caeruleatus* ZLT2)are given in Table 2. All strains produced HCN, which was confirmed by an orange colour on the filter paper. In addition, it was found that three strains (*Streptomyces mutabilis* CAR2, *Streptosporangium becharense* SG1 and *Streptomyces caeruleatus* ZLT2) were able to grow on CAS-blue agar and to produce siderophores. The seven actinobacteria grew well on colloidal chitin medium and were qualified with positive chitinolytic activity.

Indole-3-acetic acid production

The seven selected actinobacterial strains were studied for IAA production in YT broth amended with L-Tryptophan. All culture supernatants were used for extraction of indole compounds, which were revealed by the Salkowski reagent. Among the seven selected actinobacterial strains, three (*Streptomyces mutabilis* CAR2, *Streptosporangium becharense* SG1 and *Streptomyces caeruleatus* ZLT2) produced IAA. The TLC analysis demonstrated that IAA was the sole indole compound present in the culture supernatants and the same R_f values were obtained by chromatograms of authentic IAA developed with Ehmann's reagent. The amount of IAA produced varied in the range of 21–141 μg ml^{-1} and the strain *S. becharense* SG1 reached the highest level of IAA production (Table 2).

Phosphate solubilisation ability

Pikovskaya's broth amended with $Ca_3(PO_4)_2$, $FePO_4$ or $AlPO_4$ as sources of inorganic phosphate was used to highlight the phosphate solubilisation abilities of selected strains. Results given in Table 2 show that all the actinobacteria tested grew well on the three Pikovskaya media and they dissolved phosphorus from tricalcium phosphate and aluminium phosphate sources. The amount of phosphorus dissolved from $Ca_3(PO_4)_2$ in the supernatant cultures varied from 480 to 720 mg l^{-1} and the highest amount was achieved by the strain *S. becharense* SG1. The amount of phosphorus dissolved from $AlPO_4$ varied from 55 to 329 mg l^{-1}, with the strain *Streptomyces mutabilis* CAR2 attaining the highest phosphate solubilisation activity. Except for the strains *Saccharothrix longispora* MB29 and *Streptomyces neopeptinius* TLE8, all other actinobacteria dissolved phosphorus from iron phosphate.

In vivo biocontrol of Fusarium culmorum

Untreated seeds sown in infested soils (positive control) showed the highest disease severity indexes (DSI) of *F. culmorum* root rot in wheat seedlings, in both autoclaved and non-autoclaved soils (Figures 1A and 2B,C). This proves the virulence of the pathogen and the high sensitivity of the vitron variety of durum wheat.

Bacterization of wheat seeds with spores of antagonistic actinobacteria and chemical treatment with Dividend® decreased significantly ($P < 0.05$) the disease incidence, which was more noticeable in non-autoclaved than in autoclaved soil (Figures 1A and 2A). Compared to the positive control (untreated seeds in infested soils) and with reference to their antagonistic activities, the seven actinobacteria selected (*Streptomyces neopeptinius* TLE8,

Table 2. In vitro biocontrol and plant-growth-promoting traits of antagonistic actinobacteria.

Strain	Hydrogen cyanide production	Halo diameter (mm)[a]		IAA production (μg ml^{-1})[a]	Amount of dissolved phosphorus in Pikovskaya media (mg l^{-1})[a]		
		Chitinolytic activity	Siderophore production		$Ca_3(PO_4)_2$	$AlPO_4$	$FePO_4$
Streptomyces sp. strain TLE4	+	10 ± 0.7	00	00	472 ± 0.4	95 ± 0.7	80 ± 1.4
Streptomyces neopeptinius strain TLE8	+	11 ± 0.4	00	00	480 ± 0.4	110 ± 0.1	00
Streptomyces mutabilis strain CAR2	+	17 ± 0.4	20 ± 0.9	21 ± 3.1	670 ± 1.3	329 ± 3.2	220 ± 1.2
Streptomyces strain DNT4	+	08 ± 0.1	00	00	510 ± 0.3	55 ± 0.3	107 ± 0.2
Streptosporangium becharense strain SG1	+	18 ± 1.2	24 ± 0.2	141 ± 4.3	720 ± 1.3	78 ± 1.01	100 ± 0.1
Streptomyces caeruleatus strain ZLT2	+	18 ± 0.1	20 ± 0.1	38 ± 1.3	692 ± 5.1	207 ± 4.3	225 ± 0.3
Saccharothrix longispora strain MB29	+	12 ± 0.5	00	00	542 ± 0.23	221 ± 1.2	00

[a] = Standard deviation from three replicates.

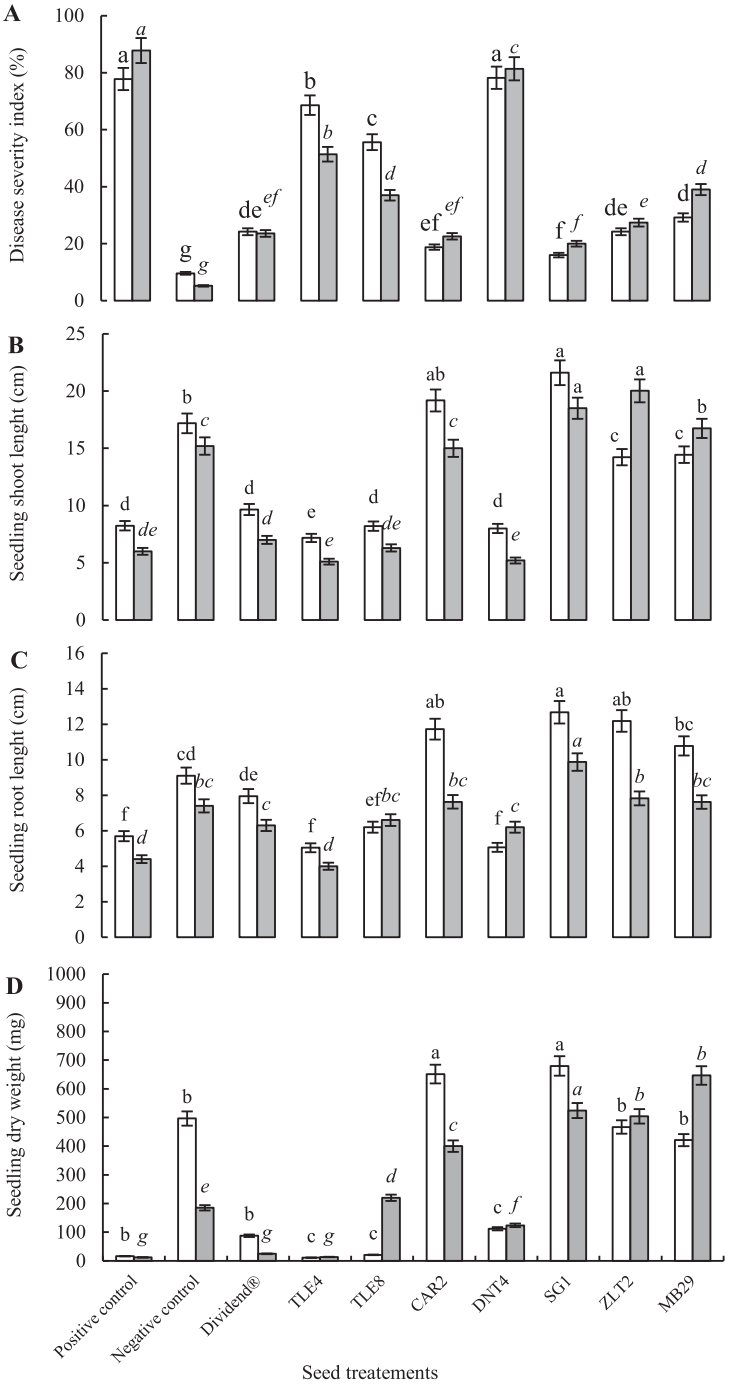

Figure 1. Effect of seed treatment with Dividend® and spore suspensions of antagonistic actinobacteria (TLE4, TLE8, CAR2, DNT4, SG1, ZLT2 and MB29) on the disease severity index (A), shoot length (B), root length (C) and seedling dry weight (D) in non-autoclaved (*white bars*) and autoclaved (*grey bars*) soils. Evaluation was made 4 weeks after planting. Bars labelled with the same letters are not significantly different according to Tukey's test at $P = 0.05$.

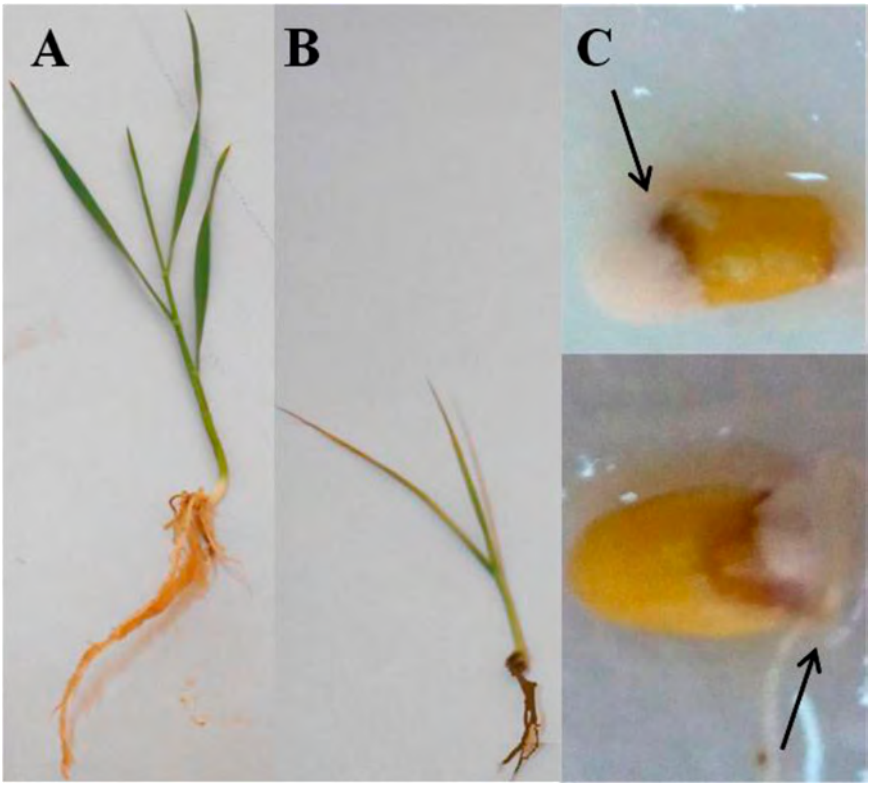

Figure 2. Healthy seedlings of wheat (cv. vitron) from seeds bacterized with spores of *Streptosporangium becharense* SG1 and grown in non-autoclaved soil infested with *F. culmorum* (LF18) (A), root rot of wheat seedlings grown in non-autoclaved soil infested with *F. culmorum* (B), and rotten seeds with mycelial growth of the fungus on the seed surface (C). Pictures were taken 10 days after planting.

Streptomyces sp. TLE4, *Streptomyces mutabilis* CAR2, *Streptomyces* sp. DNT4, *Saccharothrix longispora* MB29, *Streptosporangium becharense* SG1 and *Streptomyces caeruleatus* ZLT2) showed biocontrol effects on *F. culmorum* root rot *in vivo*. Bacterization of wheat seeds significantly ($P < 0.05$) reduced the disease severity index and the strain *S. becharense* SG1 reached the highest biocontrol potential (Figure 2A).

Compared to untreated wheat seeds in non-infected soils (negative control), the strain *S. becharense* SG1 achieved the higher effect in promoting the growth of wheat seedlings. It significantly ($P < 0.05$) increased: the shoot length, from 15.88 to 21.58 cm in non-autoclaved soil and from 15.2 to 18.5 cm in autoclaved soil (Figure 1B); the root length, from 7.11 to 12.62 cm in non-autoclaved soil and from 7.4 to 9.86 cm in autoclaved soil (Figure 1C); and the dry weight, from 0.26 to 0.7 g in non-autoclaved soil and from 0.185 to 0.524 g in autoclaved soil (Figure 1D).

Results of the PCA of data recorded in the *in vivo* assay provided a general view of biocontrol efficiency against *F. culmorum* root rot for the actinobacterial strains tested (Figure 3). The first axis (PC1) and second axis (PC2) explained 97.26% of the differences between treatments (93.84% for PC1 and 3.41% for PC2). As shown by the ANOVA results, PCA clearly divided strains into three groups. Group (A) comprised strains *Streptosporangium*

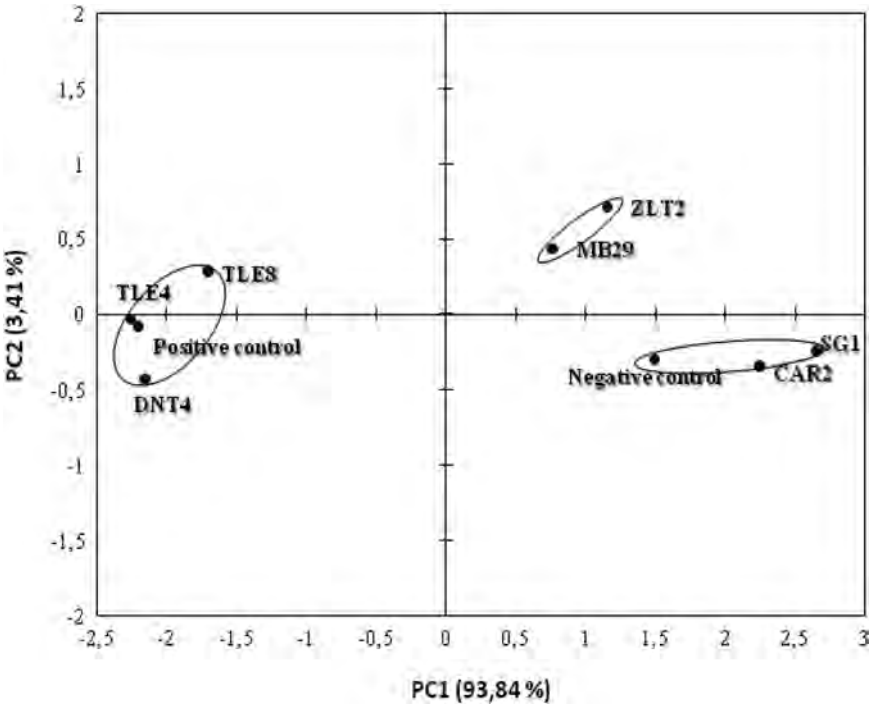

Figure 3. Principal component (PC) analysis plots (PC1 and PC2) to show the efficacy of actinobacterial strains (TLE4, TLE8, CAR2, DNT4, SG1, ZLT2 and MB29) tested for biocontrol of *Fusarium culmorum* and plant-growth-promoting effect. The control treatments correspond to untreated seeds sown in non-infested soil (negative control) or in infested soil (positive control).

becharense SG1 and *Streptomyces mutabilis* CAR2, which expressed the best biocontrol performance. Group (B) comprised MB29 (*Saccharothrix longispora*) and ZLT2 (*Streptomyces caeruleatus*). These strains showed high biocontrol performance. Group (C) comprised *Streptomyces* sp. TLE4, *Streptomyces neopeptinius* TLE8 and *Streptomyces* sp. DNT4, which were characterised by low performance in controlling *F. culmorum* root rot.

Among strains of group (A), *S. becharense* SG1 treatment exhibited the PCA values closest to the control, thus expressing the best biocontrol performance. However, strains of group (C) appeared to be unsuitable for biocontrol purposes.

Discussion

Several studies have already reported that antagonist *Streptomyces* species can be considered as active against numerous phytopathogenic fungi, such as *F. oxysporum* f. sp. *radicis-lycopersici*, *F. culmorum* and *R. solani* (Yekkour et al., 2012; Zamoum et al., 2017), and have suggested their use as biocontrol agents, or their involvement in the *in vivo* biocontrol of wheat root rot caused by *F. culmorum* (Toumatia et al., 2015). The study by El-Tarabily, Hardy GES, Sivasithamparam, Hussein, and Kurtboöke (1997) was the first to provide evidence of the utility of *Streptosporangium albidum* in the biocontrol of *Pythium coloratum*. They reported that the mechanism involved in disease reduction can be explained by the production of non-volatile antifungal compounds.

These molecules from actinobacteria may facilitate the biocontrol of plant diseases but this is not the only mechanism by which biocontrol occurs (El-Tarabily et al., 1997; Franco-Correa et al., 2010).

Our results show that all strains can produce HCN. This volatile antifungal compound can inhibit the growth of *F. culmorum* and reduce root rot disease, as noted by Aydi-Benabdallah, Jabnoun-Khireddine, Nefzi, Mokni-Tlili, and Daami-Remadi (2016). Our findings have shown that *S. becharense* SG1 isolated from Saharan soil (Chaabane Chaouch et al., 2016b) produces HCN and significantly reduces the *F. culmorum* root rot of durum wheat. Furthermore, Defago et al. (1990) suggested that HCN production worked by inducing resistance in host plants. However, results by El-Tarabily et al. (1997) showed that *Streptosporangium albidum* Furumai, Ogawa & Okuda (isolate 154) was unable to synthesise volatile antifungal compounds. However, to the best of our knowledge, this is the first report showing HCN production by a species belonging to the genus *Streptosporangium*.

Siderophores were produced by three actinobacterial strains. These low molecular weight compounds can solubilise and sequester iron from the soil (Sadeghi et al., 2012). Secretion of siderophores as a biocontrol mechanism has been reported for numerous actinobacterial genera such as *Streptomyces* and *Streptosporangium* (Jogaiah et al., 2016; Sadeghi et al., 2012). Siderophores permit the acquisition of ferric ion, thus inhibiting the growth of phytopathogens by competition for iron (Ramadan, AbdelHafez, Hassan, & Saber, 2016). The possible association of siderophore production with the biocontrol ability of actinobacteria has been reported by Cao, Qiu, You, Tan, and Zhou (2005). Our findings showing siderophore production by *S. becharense* SG1 are consistent with those of Jogaiah et al. (2016), who reported siderophore production by *Streptosporangium roseum* SJ_UOM-18-09.

All strains showed positive results for chitinolytic activity, which can be involved in the cell wall degradation of several phytopathogenic fungi. However, many authors have reported the potential of actinobacteria producing chitinase for the biocontrol of *F. oxysporum* f. sp. *radicis-lycopersici*, *F. oxysporum* f. sp. *lini*, *F. culmorum* and *Botrytis cinerea in situ* (Das, Kumar, Kumar, Solanki, & Kapur, 2017; Goudjal, Zamoum, Sabaou, Mathieu, & Zitouni, 2016).

According to our results, the strain *S. becharense* SG1 shows the best production of IAA. This phytohormone improves the growth of plants by enhancing seedling elongation, seed germination rate and dry weight (Goudjal et al., 2014). Several actinobacterial species have already been reported to produce IAA but this is the first report highlighting IAA production by a species of the genus *Streptosporangium*.

As reported by Khan et al. (2016), the solubilisation of inorganic phosphate is another mechanism by which actinobacteria play an important role in the promotion of plant growth. The overall growth of plants is affected by the availability of essential plant nutrients, such as phosphorus (P) (Hamdali, Hafidi, Virolle, & Ouhdouch, 2008). Several bacterial, fungal and actinobacterial strains have been found to be phosphate solubilising organisms (Khan, Zaidi, & Ahmad, 2014). They convert insoluble forms of phosphate, such as tricalcium phosphate ($Ca_3(PO_4)_2$), aluminium phosphate ($AlPO_4$) and iron phosphate ($FePO_4$), to soluble forms of phosphorus (Khan et al., 2014).

The strain *S. becharense* SG1 led to the highest rate of dissolved phosphorus from tricalcium phosphate. These findings are in agreement with results of Franco-Correa et al. (2010), who reported high activities of actinobacteria in the solubilisation of tricalcium

phosphate. Furthermore, Mba (1997) reported similar results for the solubilisation of inorganic phosphate by *Streptosporangium* species.

Our findings demonstrated that the surface treatment of seeds with the control chemical agent provided a marked protective effect against *F. culmorum* root rot. Toumatia et al. (2015) obtained similar results, indicating that seed treatment of wheat with a chemical fungicide, Tebuconazole, was effective in controlling *F. culmorum* disease. However, the massive use of such chemical compounds can lead to environmental pollution, which is a major worry in agricultural production (Ippolito & Nigro, 2000; Shimizu, 2011).

The strongest biocontrol potential *in vivo* was obtained by *S. becharense* SG1. This suggests that antibiosis is a factor that can be involved in biocontrol *in situ*, and that the production of HCN, chitinases and siderophores may also be effective mechanisms for controlling *F. culmorum* root rot (Franco-Correa et al., 2010). In addition, biocontrol of root rot *in vivo* may be affected by many factors besides nutrient availability, water status, soil temperature, soil morphology, pH value, and interactions with indigenous soil microbes (Dhanasekaran et al., 2005).

Our findings show that the biocontrol effect on *F. culmorum* is more marked in non-autoclaved soil, which suggests the presence of a synergic effect between our antagonistic actinobacterial strains and soil indigenous microflora. Similar results have been found by Errakhi, Bouteau, Lebrihi, and Barakate (2007), who highlighted the effect of soil microflora in controlling *Fusarium* root rot of sugar beet.

Biocontrol of plant diseases is often associated with promotion of plant growth (Franco-Correa & Chavarro-Anzalo, 2016; Shimizu, 2011). The strain *S. becharense* SG1 presented the highest growth-promoting effect in wheat seedlings. It increased the shoot length, root length and dry weight. Our results are consistent with those of Zamoum et al. (2015), who reported the efficacy of various actinobacterial strains in controlling *F. oxysporum* f.sp *radicis-lycopersici*. Furthermore, the effectiveness of species from the genus *Streptosporangium* in the biocontrol of *Pythium coloratum* and *Sclerospora graminicola* has been reported by El-Tarabily et al. (1997) and Jogaiah et al. (2016). However, as far as we know, this is the first work reporting the efficacy of *S. becharense* SG1 in the biocontrol of *F. culmorum* root rot disease. *S. becharense* is a new species of *Streptosporangium* discovered very recently by Chaabane Chaouch et al. at our laboratory (2016b) and no study of its efficacy in biocontrol has previously been carried out.

The strain *Streptosporangium becharense* SG1 showed the best results for all *in vitro* biocontrol and plant-growth-promoting attributes determined in our study. Thus, it showed the greatest effect in the biocontrol of *F. culmorum in vivo* and the highest plant-growth-promoting activities on durum wheat (*cv*. vitron). This is the first report highlighting such properties for the rhizospheric actinobacterium *S. becharense* SG1 and its promising perspectives for possible application in crop enhancement.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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