Potential of endophytic *Streptomyces* spp. for biocontrol of *Fusarium* root rot disease and growth promotion of tomato seedlings

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ABSTRACT

Sixteen endophytic actinobacteria isolated from roots of native plants were evaluated for their antagonistic potential against soil-borne phytopathogenic fungi. Among them, three strong antagonistic isolates were selected and characterised for *in vitro* plant-growth-promoting and biocontrol traits, including production of hydrogen cyanide, indole-3-acetic acid and siderophores, chitinase and β-1,3-glucanase activities, and inorganic phosphate solubilisation. In all trials, the strain *Streptomyces* sp. SNL2 revealed promising features. The selected actinobacteria were investigated for the biocontrol of *Fusarium oxysporum* f. sp. *radicis lycopersici* and for growth promotion of tomato (*Solanum lycopersicum* L. cv. Aïcha) seedlings in autoclaved and non-autoclaved soils. All seed-bacterisation treatments significantly reduced the root rot incidence compared to a positive control (with infested soil), and the isolate SNL2 exhibiting the highest protective activity. It reduced the disease incidence from 88.5% to 13.2%, whereas chemical seed treatment with Thiram® provided 14.6% disease incidence. Furthermore, isolate SNL2 resulted in significant increases in the dry weight, shoot and root length of seedlings. 16S rDNA sequence analysis showed that isolate SNL2 was related to *Streptomyces asterosporus* NRRL B-24328^T (99.52% of similarity). Its interesting biocontrol potential and growth enhancement of tomato seedlings open up attractive uses of the strain SNL2 in crop improvement.

KEYWORDS

Biocontrol; *Fusarium oxysporum* f. sp. *radicis lycopersici*; root rot; plant growth promotion; tomato; *Streptomyces*

Introduction

Fusarium oxysporum is considered to be among the most important ubiquitous phytopathogenic fungi (Aydi-Benabdallah, Jabnoun-Khireddine, Nefzi, Mokni-Tlili, & Daami-Remadi, 2016; Singh, Shin, Park, & Chung, 1999). Crown and root rot, caused by *F. oxysporum* f. sp. *radicis lycopersici* (*Frl*), is a common disease of tomato, which reduce the productivity both in open field and greenhouse. In the last decades, *Frl*

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crown and root rot disease are commonly investigated in tomato crops in Algeria (Zamoum et al., 2015).

Chemical treatments are commonly used to prevent crop losses from *Frl* but the intensive application of chemical pesticides may have an impact on the environment (De-Oliveira, Da Silva, & Van Der Sand, 2010). Furthermore, chemical fungicides are not totally effective on *Frl*, and disease symptoms remain a persistent problem (Huang, Zhang, Yong, Yang, & Shen, 2011). Consequently, there has been an increase in research aimed at finding new effective biocontrol agents and limiting the use of chemical pesticides.

Some actinobacterial strains are potential biocontrol agents for phytopathogenic fungi and the literature offers several reports of the isolation of actinobacteria of interest from the rhizosphere and also from various healthy plant tissues (Gopalakrishnan et al., 2011; Petrolini, Quaroni, Saracchi, & Sardi, 1996; Sardi et al., 1992; Verma, Singh, & Prakash, 2011). In addition, endophytic actinobacteria used in the management of soil-borne phytopathogenic fungi and/or to stimulate plant growth have been reported (El-Tarabily, Nassar, Hardy, & Sivasithamparam, 2009). Their mode of action includes the release of antifungal compounds, siderophores, hydrogen cyanide (HCN) and hydrolytic enzymes such as β-1.3-glucanase and chitinase (De-Oliveira et al., 2010; Passari et al., 2015). Actinobacteria can improve the growth of host plants by solubilising inorganic phosphate and enhancing the phosphorus absorption by the plant (Hamdali, Hafidi, Virolle, & Ouhdouch, 2008). Some of them are also reported to colonise the internal tissues of crop plants without causing deformations or disease symptoms, developing symbiotic associations and producing plant-growth regulators such as gibberellic acid and indole-3-acetic acid (IAA) (El-Tarabily et al., 2009).

In this context, we focused on the study of some native plants, which have successfully adapted to the xeric sandy soil in arid bioclimatic stage of the Algerian Sahara. The regeneration and vigorous growth of native plants, without human intervention, under stress full and drought conditions suggest the contribution of symbiotic microorganisms both in the biological protection of germinated seeds against soil pathogens and in the enhancement of the plant growth.

In the present study, we aimed to evaluate the potential of endophytic actinobacteria associated with roots of Saharan native plants as agents for the biocontrol of *Frl* and for the growth promotion of tomato cv. Aïcha seedlings.

Materials and methods

Sample collection and isolation of endophytic actinobacteria

Root samples of native plants (*Peganum harmala*, *Stipa tenacissima*, *Hammada scoparia*, *Solanum nigrum* and *Astragalus armatus*) were collected from Algerian Sahara (33°62′N; 3°03′E) in April 2013. Roots were washed in running water and surface-sterilised by immersion in ethanol (70%, v/v) for 5 min then in sodium hypochlorite solution (0.9%, w/v) for 20 min (Zamoum et al., 2015). Surface-sterilised roots were washed twice in sterile distilled water, cut into thin discs ($\approx 0.2 \times 0.5$ cm) and placed on chitin-vitamin agar (Hayakawa & Nonomura, 1987) supplemented with cycloheximide (80 mg l^{-1}) and polymyxin (25 mg l^{-1}) to suppress the growth of fungi and Gram-negative bacteria, respectively (Aouiche et al., 2012). The plates were incubated for 21 days at 30°C. Colonies

of actinobacteria were then purified on International *Streptomyces* Project (ISP) medium 2 (Shirling & Gottlieb, 1966).

To prove the validity of the surface-sterilisation protocol, 0.3 ml aliquot of the washing water of the surface-sterilised roots was inoculated on chitin-vitamin agar (supplemented with cycloheximide and polymyxin). Furthermore, plugs (6 mm in diameter) from actinobacterial cultures were subjected to the same surface-sterilisation treatments and inoculated on the same medium. Plates were then incubated for 14 days at 30°C and recorded for actinobacterial growth (Zamoum et al., 2015).

Taxonomic characterisation

The actinobacterial isolates were streaked on ISP2, ISP3 and ISP4 media (Shirling & Gottlieb, 1966) and incubated for 14 days at 30°C. The morphology of colonies, the colour of substrate and aerial mycelia, diffusible pigment and the morphology of the spore chains were evaluated as described by Goodfellow and Simpson (1987).

In order to determine the isomeric type of diaminopimelic acid (DAP), chemical analysis by paper chromatography of cell-wall hydrolysates was carried out. The strains belonging to the genus *Streptomyces* had cell-wall types with isomer ll-DAP and the remaining spore-forming actinobacteria contained isomer dl-DAP (Goodfellow & Simpson, 1987).

Antagonistic activity of endophytic actinobacteria

The streak method (Boubetra et al., 2013) was used to evaluate the antagonistic activities of actinobacteria against three soil-borne phytopathogenic fungi from our laboratory collection (LBSM, Ecole Normale Supérieure de Kouba, Alger, Algeria): *Frl* (F31), *Fusarium solani* (F22) and *F. oxysporum* f. sp. *albedinis* (F39). Actinobacteria were first cultivated separately in straight lines on ISP2 plates, and incubated for 1 week at 30°C. After that, target fungi were seeded in streaks perpendicular to those of actinobacteria cultivation and plates were incubated again for 5 days at 25°C. The antifungal activity was determined by measuring the distance of inhibition between actinobacteria and fungal colony margins.

Determination of biocontrol and plant-growth-promotion traits

HCN production

HCN production was highlighted by growing isolates in Bennett medium supplemented with glycine (4.4 g l^{-1}). A Whatman filter paper was flooded with 0.5% picric acid in 2% sodium carbonate for 1 min and stuck underneath the Petri dish lids. The plates were sealed with parafilm and incubated for 1 week at 30°C. The presence of an orange to red colour on the Whatman paper margins indicates a positive result for HCN production (Passari et al., 2015).

Siderophore production

The production of siderophore was evaluated on chrome azurol agar (CAS) plates (Hu & Xu, 2011). Six millimetre plugs from actinobacteria cultures were inoculated on the medium and incubated for 1 week at 30°C. The production of siderophores was revealed by the presence of orange haloes surrounding the actinobacterial colonies.

Chitinase and β-1,3-glucanase activities

The chitinase activity was performed using colloidal chitin (CC) medium (Gonzalez-Franco, Deobald, Spivak, & Crawford, 2003). Actinobacterial isolates were spot inoculated onto the medium and incubated for 5 days at 30°C. Activity of chitinase was determined by evaluating the size of the hydrolytic haloes surrounding the colonies of actinobacteria.

The β-1,3-glucanase activity was highlighted by the method of Sadeghi et al. (2012). Actinobacterial isolates were cultured in tryptic soy broth amended with CC (1%, w/v) on a rotary shaker (30°C, 200 rpm) for 5 days. The supernatant cultures were collected by centrifugation (10,000 *g*, 10 min) and 1 ml from each culture filtrate was added with 0.1 ml of laminarin solution (2%, w/v) in 0.2 M acetate buffer (pH 5.4). The reaction was carried out at 40°C for 1 h before being stopped by adding 3 ml of DNS (3,5-dinitro-salicylic acid) to the mixture and kept at boiling for10 min. The development of dark red colour indicated a positive activity of β-1,3-glucanase.

IAA production

One-millilitre aliquots from the spore suspensions ($\approx 10^6$ CFU ml^{-1}) of actinobacterial isolates were inoculates into 250-ml Erlenmeyer flasks containing 50 ml of Yeast extract-Tryptone (YT) broth (Khamna, Yokota, Peberdy, & Lumyong, 2010) amended with l-tryptophan (5 mg ml^{-1}). Flasks were cultured for 5 days on a rotary shaker (200 rpm, 30°C). The liquid cultures were then centrifuged at 4000 *g* for 30 min and the production of IAA was revealed by mixing 2 ml of the supernatant with 4 ml of Salkowski reagent (1 ml of $FeCl_3$ solution (0.5 M) in 49 ml of $HClO_4$ solution (35%, w/v)). The IAA production was highlighted by the appearance of a pink colour after 30 min in a dark room. Optical density was measured by spectrophotometer at 530 nm and the quantity of IAA was calculated using a pure IAA standard graph (Passari et al., 2015).

Phosphate solubilisation

The experiments were conducted in 500-ml Erlenmeyer flasks containing 100 ml of Pikovskaya broth (Pikovskaya, 1948) containing an insoluble phosphate source ($Ca_3(PO_4)_2$, $AlPO_4$ or $FePO_4$) at a concentration of 5 g l^{-1}. Flasks were inoculated by 1 ml aliquot of the actinobacterial spore suspensions ($\approx 10^6$ CFU ml^{-1}) and cultured for 1 week on a rotary shaker (200 rpm, 30°C). The supernatant cultures were harvested by centrifugation (10,000 *g*, 10 min) and were used to evaluate the soluble phosphate. The rate of dissolved phosphate was determined by the spectrophotometric molybdenum-blue method (Liu et al., 2014).

Biocontrol of F. oxysporum *f. sp.* radicis lycopersici

The potential of the strong antagonistic actinobacteria in the biocontrol of *Frl* and their role on the growth promotion of tomato (*Solanum lycopersicum* L. cv. Aïcha) seedlings were tested in a sandy soil sampled from a Saharan tomato field (33°62′N; 2°91′E) infested with the *Frl*. The chemical, textural and biological properties of soil were as follows: pH 6.6; organic matter 0.82%; C/N 9.6, potash 0.23‰; phosphate 0.08‰; $CaCO_3$ 1.0%; sand 75%, clay 14%, silt 11%; aerobic bacteria 1.8×10^8 CFU g^{-1} of soil and total fungal count 4.3×10^4 CFU g^{-1} of soil.

The biocontrol trials were performed both in autoclaved and non-autoclaved soils. Sterilisation was carried out by autoclaving soil samples (120°C for 60 min) thrice on 3 days.

Surface-sterilisation of tomato seeds was performed by soaking in ethanol (70%, v/v) for 3 min, in hypochlorite solution (0.9%, w/v) for 4 min followed by washing thrice in sterile distilled water. After that, surface-sterilised seeds were separately bacterised by dipping for 30 min in the suspensions of antagonistic actinobacteria ($\approx 10^6$ CFU ml^{-1}). Bacterised seeds were then dried under a microbiological laminar flow hood and seeded in the same day. Spores of actinobacteria on the bacterised seeds were estimated by dilution plating on ISP2 medium. Actinobacterial spores reached $\approx 8 \times 10^6$ CFU g^{-1} bacterised seeds.

Plastic pots (10 cm in diameter × 12 cm high) filled with autoclaved (or non-autoclaved) soils were watered with 100 ml of the *Frl* spore suspension ($\approx 10^3$ CFU ml^{-1}), or 100 ml of sterile distilled water in the case of non-infested pots. All pots were then covered with opaque polyethylene film and stored at room temperature for 1 week to favour the growth of the pathogen (Zamoum et al., 2015). The density of *Frl* in the infested soil was evaluated at $\approx 1.2 \times 10^4$ CFU g^{-1}.

Four treatments were conducted in the biocontrol assay: (1) untreated seeds sown in non-infested pots (negative control); (2) untreated seeds sown in infested pots to evaluate the varietal sensitivity (positive control); (3) the biocontrol potential of each antagonistic actinobacteria was evaluated by sowing bacterised seeds in infested pots and (4) sterilised seeds were treated with a chemical fungicide, Thiram® 75WP, as recommended by the manufacturer (mixing 10 g of the surface-sterilised seeds for 3 min in 0.08 g of Thiram® 75WP wetted with 10 ml of sterile distilled water and drying for 2 h under a microbiological laminar flow hood), before being seeded in infested pots.

Each treatment was conducted with 10 replicates and 6 tomato seeds were sown per pot. Pots were then deposited in a greenhouse (23–26°C, 15 h/9 h light/dark) in a fully randomised complete block design and were watered daily with tap water (10 ml per pot). Biocontrol experiments were conducted twice to ensure reproducibility.

After 6 weeks of culture, the disease incidence was assessed using a 5-class scale (Zamoum et al., 2015). The root and shoot lengths, and the dry weight were assessed for healthy seedlings.

Identification of the isolate SNL2

Molecular identification of the isolate SNL2 was carried out by the 16S rDNA gene sequence analysis. Genomic DNA was extracted by to the CTAB method and the 16S rDNA was amplified by the PCR method (Boubetra et al., 2013). The genomic sequence was determined by the Beckman Coulter Genomics Company (Tekeley, UK). The 16S rDNA sequence obtained was deposited in the GenBank data library and was compared for similarity with sequences of reference species contained in the public sequence databases, using EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012).

Statistical analysis

All *in vitro* experiments were carried out with three replicates. Biocontrol experiments were conducted twice with 10 replicates for each treatment. Data were subjected to

ANOVA analysis and results represent the mean ± standard deviation. Significant differences between means were compared using Fisher's protected LSD test at $P = 0.05$.

Results

Actinobacterial isolates and determination of genera

Sixteen actinobacterial strains were isolated from the five Saharan native plants. Aliquots of the sterile distilled water used to wash surface-sterilised roots failed to grow any actinobacterial colonies on chitin-vitamin medium. Furthermore, all cultures of actinobacterial isolates treated with the surface-sterilisation protocol were unable to grow on this medium. This demonstrates the effectiveness of the sterilisation protocol to eradicate rhizospheric actinobacteria from the surface of roots. Therefore, all the actinobacteria isolated were indeed endophytic.

Morphological analysis showed the presence of aerial mycelia for all the 16 isolates cultured on ISP2, ISP3 and ISP4 media. On the basis of their spore-mass colour, isolates were ranked in the grey group (12 isolates) or yellow group (3 isolates) (Table 1).

Micromorphological analysis showed that actinobacteria from the grey group (75%) produced spore chains (type *Spira*). However, those from the yellow group (19%) were characterised by the production of right and flexuous spore chains (type *Rectiflexibilis*).

The chemotaxonomic analysis revealed the presence of isomer ll-DAP in the cell-wall hydrolysates of 15 isolates. Based on this taxonomical characteristic, these actinobacteria were classified in the *Streptomyces* genus. Nevertheless, one isolate had dl-DAP isomer and was considered as a non-*Streptomyces* strain (Table 1).

Antagonistic activities

Of the 16 actinobacterial isolates, 9 (56.2%) exhibited antagonistic activity against at least two of the three phytopathogenic tested fungi. Three isolates showed positive antagonistic activities against all the tested fungi (Table 2).

Table 1. Morphological and chemical characteristics of endophytic actinobacteria.

	Colour of						
Host plant	Spore mass	Diffusible pigment	Colour group	Spore-chain type	DAP isomer	Genus	Number of isolates
P. harmala	Brownish grey	Without	Grey	S	ll	*Streptomyces*	2
	White	Without	–	–	dl	Non-*Streptomyces*	1
S. tenacissima	Grey	Without	Grey	S	ll	*Streptomyces*	4
	Greenish grey	Without	Grey	S	ll	*Streptomyces*	1
H.a scoparia	Grey	Without	Grey	S	ll	*Streptomyces*	2
S. nigrum	Brownish grey	Without	Grey	S	ll	*Streptomyces*	2
A. armatus	Greyish yellow	Without	Yellow	RF	ll	*Streptomyces*	3
	Grey	Without	Grey	S	ll	*Streptomyces*	1
Total							16

Note: S, Spira; RF, Rectiflexibilis.

Table 2. Antagonistic activities of endophytic actinobacteria against phytopathogenic fungi.

		Antagonistic activity (zone of inhibition in mm)[a]		
Host plant	Isolate	*F. oxysporum* f. sp. *radicis lycopersici* (LF31)	*F. solani* (LF22)	*F. oxysporum* f. sp. *albedinis* (LF39)
P. harmala	PHL1	0	0	18 ± 1.8
	PHL2	0	18 ± 1.7	0
	PHL3	0	12 ± 2.2	10 ± 0.6
S. tenacissima	STL1	0	17 ± 0.6	10 ± 1.6
	STL2	0	18 ± 0.9	0
	STL3	0	15 ± 1.6	05 ± 1.0
	STL4	0	00	18 ± 1.2
	STL5	0	32 ± 1.2	32 ± 1.9
H. scoparia	HSL1	0	0	16 ± 1.4
	HSL2	12 ± 1.2	0	0
S. nigrum	SNL1	24 ± 0.9	14 ± 1.8	10 ± 1.6
	SNL2	32 ± 2.1	28 ± 1.9	29 ± 0.9
A. armatus	AAL1	0	20 ± 1.6	12 ± 1.8
	AAL2	21 ± 1.5	34 ± 2.1	32 ± 2.2
	AAL3	0	0	0
	AAL4	0	16 ± 1.6	10 ± 2.4

[a]Average ± standard deviation from three replicates.

The strongest antagonistic activities were found against *F. solani* and *F. oxysporum* f. sp. *albedinis*. However, the majority of isolates (81.2%) failed to inhibit the mycelial growth of *Frl* or inhibited it only moderately. Nevertheless, three isolates showed strong antagonistic activities against *Frl* (inhibition zone > 20 mm) and the highest zone of inhibition was found for the isolate SNL2.

HCN and siderophore production, and hydrolytic enzymes activity

The three selected antagonistic actinobacteria (SNL1, SNL2 and AAL2) were studied for HCN and siderophore production abilities. HCN production was detected in two isolates, SNL1 and SNL2. These two actinobacteria were found to be able to release siderophores on CAS medium. However, the isolate AAL2 failed to produce HCN or siderophores. The results also showed positive activities of chitinase and β-1,3-glucanase for the three actinobacteria (Table 3).

IAA production and phosphate solubilisation ability

All three actinobacteria (SNL1, SNL2 and AAL2) were positive for the production of IAA in YT broth, with isolate SNL2 producing the maximum amount of IAA (Table 3).

The three actinobacteria tested dissolved tricalcium phosphate (TCP) (Table 3), two isolates released phosphorus from aluminium phosphate (AlP) source and all isolates

Table 3. HCN and siderophore production, and hydrolytic enzymes activities of antagonistic actinobacteria.

		Halo diameter (mm)[a]		β-1,3-Glucanase activity	IAA production (μg ml^{-1})[a]	Amount of dissolved P (mg l^{-1})[a] in Pikovskaya media		
Isolate	HCN	Siderophore	Chitinolytic activity			Pikovskaya (TCP)	Pikovskaya (AlP)	Pikovskaya (FeP)
SNL1	+	16 ± 1.4	24 ± 2.1	+	22.6 ± 9	431 ± 15	132 ± 21	0
SNL2	+	20 ± 0.9	22 ± 1.9	+	99.7 ± 11	662 ± 11	226 ± 14	0
AAL2	–	0	20 ± 1.6	+	17.3 ± 4	558 ± 8	0	0

[a]Average ± standard deviation from three replicates.

failed to dissolve iron phosphate (FeP). A quantitative estimation of the phosphorus released showed that the isolate SNL2 reached the highest phosphate solubilisation activities. However, all isolates were unable to solubilise FeP (Table 3).

Biocontrol of F. oxysporum *f. sp.* radicis lycopersici

The results of the biocontrol potential and plant-growth-promoting effect of the three antagonistic actinobacteria (SNL1, SNL2 and AAL2) are given in Figure 1. High disease incidences of the *Frl* root rot of seedlings in both autoclaved (90.3%) and non-autoclaved (88.5%) soils were obtained for the positive control. Symptoms of the root rot disease were observed in germinated seeds and young seedlings, leading to the roots desiccation and the seedlings death (Figure 2).

Chemical treatment with Thiram® 75WP and bacterisation of tomato seeds with spores of antagonistic actinobacteria reduced significantly ($P < 0.05$) the incidence of root rot, which was relatively lower in non-autoclaved soil (Figure 1(a)). Chemical treatment showed a strong protective activity against the *Frl* root rot. Non-significant differences were showed between the treatment with Thiram® and the bacterisation of tomato seeds with spores of the isolate SNL2, which reached the highest biocontrol effect in both autoclaved and non-autoclaved soils. The healthy seedling rate obtained in the negative control was 89.5%. For the seed treatment with Thiram® and the seed bacterisation with spores of SNL2 isolate, the rates were 77.3% and 78.8%, respectively. Based on their biocontrol potentials, the isolates AAL2 and SNL1 were ranked in second and third positions, respectively.

Seed bacterisation with spores of the isolate SNL2 showed the greatest effect in enhancing growth of tomato seedling (Figure 3). Compared to the negative control, this treatment significantly increased ($P < 0.05$) the dry weight of seedlings from 0.9 to 1.1 g (Figure 1(b)), the shoot length from 9.9 to 11.5 cm (Figure 1(c)) and the root length from 4.9 to 6.2 cm (Figure 1(d)) in non-autoclaved soil. For all the remaining treatments, bacterisation of tomato seeds failed to improve the growth of tomato seedlings significantly. The dry weight of seedlings varied from 0.7 to 1.0 g, the seedling shoot length from 9.9 to 11.5 cm and the seedling root length from 4.8 to 5.5 cm (Figure 1(b)–(d)).

Taxonomical position of the strain SNL2

On the basis of its biocontrol and plant-growth-promotion properties, the isolate SNL2 was selected for molecular identification. The 16S rDNA sequence has been deposited in the GenBank (accession number KC414014). The sequence was aligned with those of *Streptomyces* reference species available in GenBank database, which confirmed that the isolate SNL2 belonged to the genus *Streptomyces*. The identity level was 99.52% with *Streptomyces asterosporus* NRRL B-24328^{T}.

Discussion

Several reports of the isolation of endophytic actinobacteria from roots, stems and leaves of various crop or native plant species can be found in the literature (Petrolini et al., 1996; Sardi et al., 1992; Shimizu, 2011). Some actinobacterial species have also been reported as

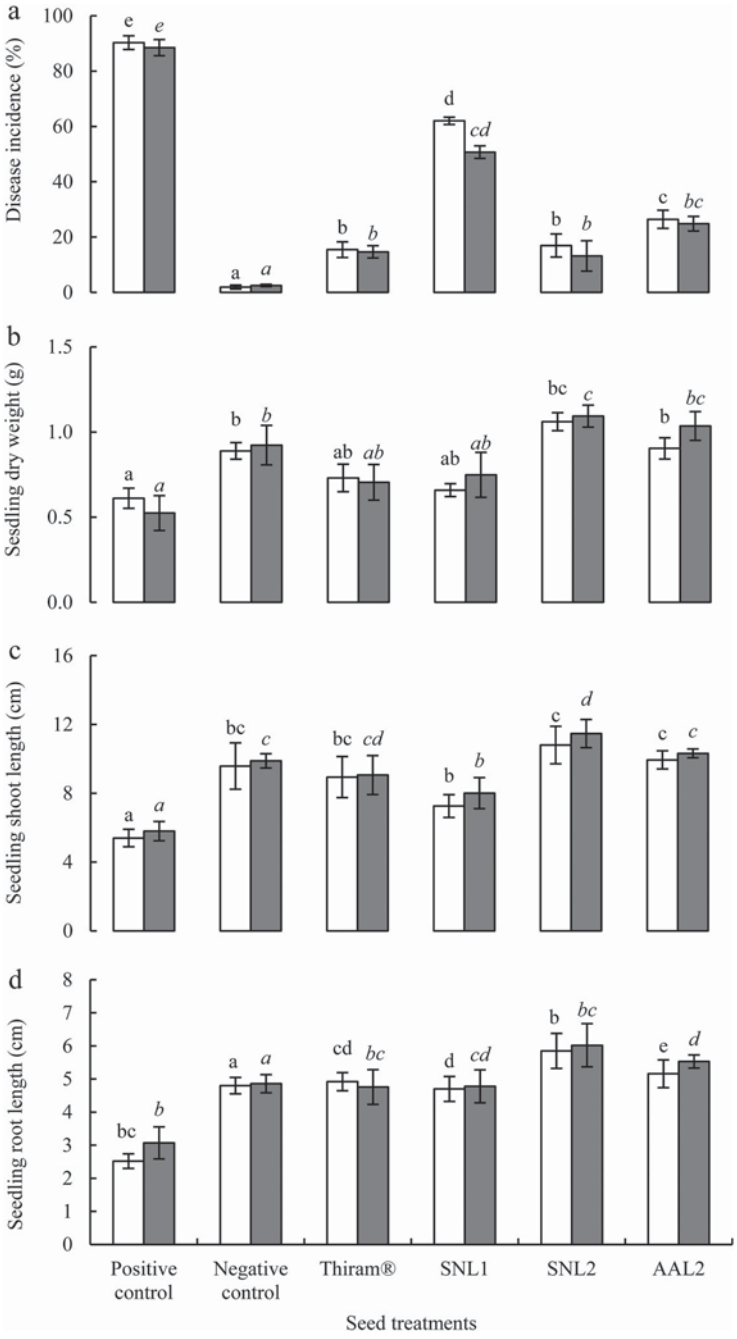

Figure 1. Effect of seed treatment with Thiram® and spore suspensions of antagonistic actinobacteria (SNL1, SNL2 and AAL2) on the disease incidence (a), seedling dry weight (b), shoot length (c) and root length (d) in autoclaved (*white bars*) and non-autoclaved (*grey bars*) soils. The control treatments correspond to untreated seeds sown in infested pots (positive control) or in non-infested pots (negative control). Evaluation was made 6 weeks after planting. Bars labelled with the same letters are not significantly different according to Fisher's protected LSD test at $P = 0.05$.

stimulators for growth of plants and as potential biocontrol agents of various phytopathogenic fungi (Huang et al., 2011; Toumatia et al., 2016; Verma et al., 2011; Zamoum et al., 2015). Furthermore, the efficacy of isolated actinobacteria in the biocontrol of the *Frl* root rot disease has been highlighted (Aydi-Benabdallah et al., 2016; Saidi et al., 2009; Zamoum et al., 2015). In addition, commercial formulations of *Streptomyces* spp. to control phytopathogenic *Fusarium* spp., such as *Streptomyces griseoviridis* strain K61 (Mycostop®) and *Streptomyces lydicus* strain WYEC108 (Actinovate®), have been registered (Lahdenperä, Simon, & Uoti, 1991; Young & Crawford, 1995).

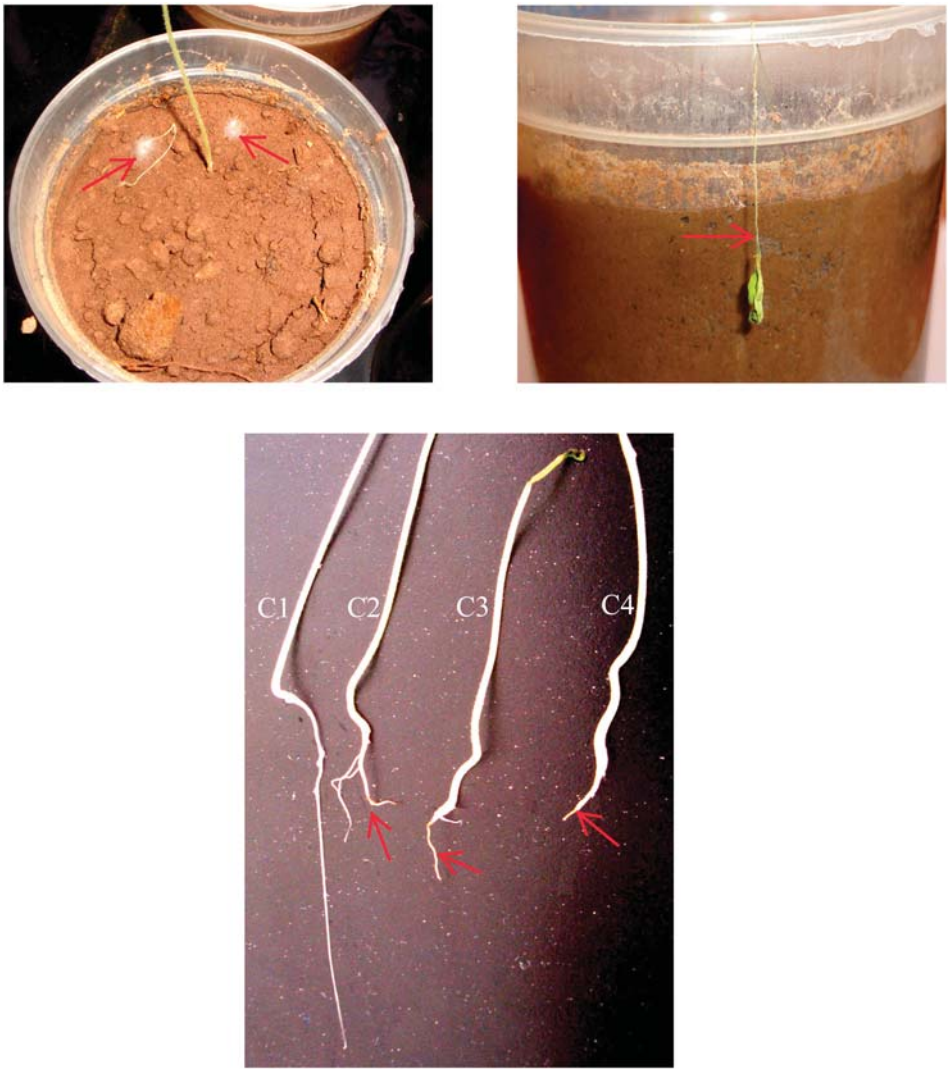

Figure 2. Symptoms of the root rot disease showed on seedlings of tomato cv. Aïcha grown in the *F. oxysporum* f. sp. *radicis lycopersici* infested soil. Arrows indicate the mycelial growth of the fungi on the soil surface (a), damping-off of tomato seedling obtained from an untreated seed (b), and the fungal root rot on seedlings (c2, c3 and c4) obtained from untreated seeds compared to a healthy root obtained from seed-bacterisation with spores of *S. asterosporus* strain SNL2 (c1). The pictures were taken after 2 weeks of culture under standard greenhouse conditions.

In the present study, 16 actinobacterial strains of were isolated from the roots of native Saharan plants. The protocol used in the surface-sterilisation of roots was found to be efficient to eliminate rhizospheric microbes, which proved that all actinobacteria were isolated from the root interior tissues.

Except for the strain PHL3 isolated from roots of *P. harmala*, all endophytic actinobacteria belonged to the genus *Streptomyces*. These results are supported with several reports that highlighted the dominance of the genus *Streptomyces* in roots of some native plants (Passari et al., 2015; Petrolini et al., 1996; Sardi et al., 1992).

In this study, three endophytic actinobacteria isolated from roots of *S. nigrum* (SNL1 and SNL2) and *A. armatus* (AAL2) from the Algerian Sahara showed strong antagonistic activities towards the mycelial growth of *Frl*. Antagonistic isolates were selected to highlight, firstly, their biocontrol and plant-growth-promoting properties. Furthermore, potential for biocontrol of *Frl* and for growth improvement in tomato seedlings was assessed to select the most interesting isolate.

The isolate SNL2 showed strong antagonistic activities against all phytopathogenic tested fungi, especially *Frl*. These results are in agreement with several *Streptomyces* species, such as *S. caeruleatus* strain ZL2 (Zamoum et al., 2015) and *S. mutabilis* strain IA1 (Toumatia et al., 2016), which showed antifungal activities against *Fusarium* species.

Production of antifungal compounds is an advantage in the biocontrol of phytopathogenic fungi since antifungal compounds can easily diffuse in the soil and consequently

Figure 3. Healthy seedlings of tomato cv. Aïcha obtained by sowing untreated seeds in non-infested soil as negative control (left) and bacterised seeds with spores of *S. asterosporus* strain SNL2 (right) sowed in infested soil with spores of *F. oxysporum* f. sp. *radicis lycopersici*. The picture was taken after 2 weeks of culture under standard greenhouse conditions.

direct contact between fungi and the antagonistic *Streptomyces* is not necessary (De-Oliveira et al., 2010; Shimizu, 2011). *Streptomyces* spp. producing antifungal compounds are already involved in the biocontrol of phytopathogenic *Fusarium* species (Yekkour et al., 2012).

Certain endophytic *Streptomyces* species have been reported to produce HCN, a volatile antifungal compound, which contributes in suppression of *Fusarium* disease (Aydi-Benabdallah et al., 2016; Passari et al., 2015). Here, we detected two *Streptomyces* isolates that were positive for HCN production. Furthermore, production of HCN is recognised as the main biocontrol mechanism of *Pseudomonas fluorescens* (Shimizu, 2011).

Siderophore production is another biocontrol feature of antagonistic *Streptomyces* species. The siderophore production plays an important role in antagonism activities. Furthermore, actinobacteria producing siderophores may help the plant to take up iron from the soil (Sadeghi et al., 2012; Verma et al., 2011). Among the three antagonistic actinobacteria tested, two isolates (SNL1 and SNL2) produced siderophores on CAS medium and the highest production was shown with isolate SNL2. These findings are supported with results of Cao, Qiu, You, Tan, and Zhou (2005) who reported the role of endophytic *Streptomyces* sp. S96 producing siderophores in the antagonistic effect on *F. oxysporum* f. sp. *cubense*.

Chitinase and β-1,3-glucanase from *Streptomyces* involved in the destruction of fungal cell walls are an important mechanisms in the antagonistic activities against soil-borne pathogenic fungi. Actinobacteria producing chitinase and β-1,3-glucanase are often used in biocontrol processes and are commonly used for the formulation of biopesticides (Gonzalez-Franco et al., 2003; Kumaran, Deivasigamani, & Uttara, 2012). As reported for *Streptomyces* sp. strain 385, which suppressed *Fusarium* wilt of cucumber (Singh et al., 1999), all antagonistic *Streptomyces* showed positive results for chitinase and β-1,3-glucanase activities. The potential role of *Streptomyces* producing chitinase and β-1,3-glucanase in the biocontrol of *Frl* root rot have been highlighted (Shimizu, 2011).

Various endophytic *Streptomyces* species, such as *S. rimosus*, *S. viridis* and *S. olivaceoviridis*, have been reported to produce IAA, which improves the growth of the host plants by increasing germination of seeds, root elongation and root dry weight (El-Tarabily et al., 2009; Khamna et al., 2010). Our results showed that the three isolates tested produced IAA in YT broth, with the isolate SNL2 showing the best production of IAA. This was higher than the production reported for *S. viridis* strain CMU-H09 (28.5 μg ml^{-1}) (Ruanpanun, Tangchitsomkid, Hyde, & Lumyong, 2010) and for *S. caeruleatus* strain ZL2 (64 μg ml^{-1}) (Zamoum et al., 2015).

Inorganic phosphate solubilisation is another mechanism by which actinobacteria play an interesting role in the enhancement of plant growth (Hamdali et al., 2008). In our findings, the three actinobacteria tested dissolved TCP in the Pikovskaya's broth. Only two isolates (SNL1 and SNL2) grew and released phosphorus from an AlP source. However, all isolates failed to grow depending to FeP as phosphorus source. The isolate SNL2 showed the highest amount of dissolved phosphate from TCP, which, although a potentially insoluble phosphate, is not hard to dissolve compared with other forms of mineral phosphate (AlP and FeP). These results agree those of Liu et al. (2014), who reported low activities of phosphate-solubilising bacteria in the solubilisation of FeP. However, the phosphate solubilisation activities of the isolate SNL2 were lower than those reported for *S. mutabilis* strain CA2 (Zamoum et al., 2015), which dissolved all the three forms of mineral phosphate.

Biocontrol of *Frl* showed a high rate of disease incidence for the positive control in both autoclaved and non-autoclaved soils. This proves the high sensitivity of tomato cv. Aïcha to the pathogen, which was very virulent. Chemical seed treatment with Thiram® decreased significantly the incidence of root rot by over 80%. These findings are in agreement with results of Zamoum et al. (2015), who highlighted the efficacy of chemical treatment of seeds in the suppression of *Frl* root rot disease. However, repeated and abusive uses of chemical pesticides, herbicides and fertilisers may cause environmental pollution problems, which are a major concern in crop production. In such situations, biocontrol methods to manage such disease could be an appropriate solution (Passari et al., 2015).

The three actinobacteria selected showed biocontrol effects on *Frl*. Compared to the positive control; all treatments of seed bacterisation significantly decreased the root rot incidence (by over 43%) and the strain SNL2 was the most strong among them. Similarly, *Streptomyces caeruleatus* strain ZL2 (Zamoum et al., 2015) and *Pseudomonas* sp. strain S85 (Aydi-Benabdallah et al., 2016) reduced significantly the root rot disease of tomato seedlings caused by phytopathogenic *Fusarium* species. The biocontrol efficiency can be affected by many factors, including environmental conditions such as morphology of soil, temperature, pH, nutrient availability, water status and interactions with indigenous soil microorganisms (Shimizu, 2011).

The interaction of indigenous microorganisms of soil in the biocontrol of *Frl* showed a positive effect. Similar results have been highlighted by Zamoum et al. (2015). They suggest the presence of antagonistic microbes within indigenous microflora that play a synergic effect with the antagonistic *Streptomyces* species.

Biocontrol activities of actinobacteria are often associated with promotion of plant growth (El-Tarabily et al., 2009; Passari et al., 2015; Verma et al., 2011; Toumatia et al., 2016). The isolate SNL2 achieved the highest effect in promoting growth of tomato seedlings and it significantly increased the root length, shoot length and dry weight of tomato seedlings in comparison with results of the negative control. These findings agree those showed by *Streptomyces* sp. strain C (Sadeghi et al., 2012) and *S. neopeptinius* strain TL7 (Goudjal et al., 2016), which significantly improved the growth of tomato seedlings.

The strain *S. asterosporus* SNL2 yielded the greatest biocontrol effect on *Frl* and the highest growth-promotion activity on tomato seedlings. Furthermore, this endophytic strain, isolated from roots of *S. nigrum*, showed positive results for all the biocontrol and plant-growth-promoting traits determined. Such beneficial properties open up promising perspectives for its possible exploration in the biocontrol field.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

Aouiche, A., Sabaou, N., Meklat, A., Zitouni, A., Bijani, C., Mathieu, F., & Lebrihi, A. (2012). Saccharothrix sp. PAL54, a new chloramphenicol-producing strain isolated from a Saharan soil. *World Journal of Microbiology and Biotechnlogy*, *28*, 943–951.

Aydi-Benabdallah, R., Jabnoun-Khireddine, H., Nefzi, A., Mokni-Tlili, S., & Daami-Remadi, M. (2016). Endophytic bacteria from *Datura metel* for plant growth promotion and bioprotection against *Fusarium* wilt in tomato. *Biocontrol Science and Technology*, *26*, 1139–1165.

Boubetra, D., Sabaou, N., Zitouni, A., Bijani, C., Lebrihi, A., & Mathieu, F. (2013). Taxonomy and characterization of new antibiotics produced by *Saccharothrix* SA198 isolated from a Saharan soil. *Microbiological Research*, *168*, 223–230.

Cao, L., Qiu, Z., You, J., Tan, H., & Zhou, S. (2005). Isolation and characterization of endophytic streptomycetes antagonists of *Fusarium* wilt pathogen from surface-sterilized banana roots. *FEMS Microbiology Letters*, *247*, 147–152.

De-Oliveira, M. F., Da Silva, M. G., & Van Der Sand, S. T. (2010). Anti-phytopathogen potential of endophytic actinobacteria isolated from tomato plants (*Lycopersicon esculentum*) in southern Brazil, and characterization of *Streptomyces* sp. R18, a potential biocontrol agent. *Research in Microbiology*, *161*, 565–572.

El-Tarabily, K. A., Nassar, A. H., Hardy, G. E. S. J., & Sivasithamparam, K. (2009). Plant growth promotion and biological control of *Pythium aphanidermatum* a pathogen of cucumber, by endophytic actinomycetes. *Journal of Applied Microbiology*, *106*, 13–26.

Gonzalez-Franco, A. C., Deobald, L. A., Spivak, A., & Crawford, D. L. (2003). Actinobacterial chitinase-like enzymes: Profiles of rhizosphere versus non-rhizosphere isolates. *Canadian Journal of Microbiology*, *49*, 683–698.

Goodfellow, M., & Simpson, K. E. (1987). Ecology of streptomycetes. *Frontier in Applied Microbiology*, *2*, 97–125.

Gopalakrishnan, S., Pande, S., Sharma, M., Humayun, P., Kiran, B. K., Sandeep, D., … Rupela, O. (2011). Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of *Fusarium* wilt of chickpea. *Crop Protection*, *30*, 1070–1078.

Goudjal, Y., Zamoum, M., Meklate, A., Sabaou, N., Mathieu, F., & Zitouni, A. (2016). Plant-growth-promotiong potential of endosymbiotic actinobacteria isolated from sand truffles (*Terfezia leonis* Tul.) of the Algerian Sahara. *Annals of Microbiology*, *66*, 91–100.

Hamdali, H., Hafidi, M., Virolle, M. J., & Ouhdouch, Y. (2008). Growth promotion and protection against damping-off of wheat by two rock phosphate solubilizing actinomycetes in a P-deficient soil under greenhouse conditions. *Applied Soil Ecology*, *40*, 510–517.

Hayakawa, M., & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *Journal of Fermentation Technology*, *65*, 501–509.

Hu, Q. P., & Xu, J. G. (2011). A simple double-layered chrome azurol S agar (SD-CASA) plate assay to optimize the production of siderophores by a potential biocontrol agent *Bacillus*. *African Journal of Microbiology Research*, *5*, 4321–4327.

Huang, X., Zhang, N., Yong, X., Yang, X., & Shen, O. (2011). Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with *Bacillus pumilus* SQR-N43. *Microbiological Research*, *167*, 135–143.

Khamna, S., Yokota, A., Peberdy, J. F., & Lumyong, S. (2010). Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. *Euro Asian Journal of Biological Science*, *4*, 23–32.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., … Chun, J. (2012). Introducing EzTaxon-e: A prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *International Journal of Systematic and Evolutionary Microbiology*, *62*, 716–721.

Kumaran, S., Deivasigamani, B., & Uttara, V. (2012). *Chitinase application review*. Saarbrücken, Germany: Lambert Book Publishing House.

Lahdenperä, M. L., Simon, E., & Uoti, J. (1991). Mycostop – a novel biofungicide based on *Streptomyces* bacteria. In A. B. R. Beemster, G. J. Bollen, M. Gerlagh, M. A. Ruissen, B.

Schippers, & A. Temple (Eds.), *Biotic interactions and soil-borne disease* (pp. 258–263). Elsevier: Amsterdam.
Liu, F., Liu, H., Zhou, H., Dong, Z., Bai, X., Bai, P., & Qioa, J. (2014). Isolation and characterization of phosphate-solubilizing bacteria from betel nut (*Areca catechu*) and their effects on plant growth and phosphorus mobilization in tropical soils. *Biology and Fertility of Soils*, *50*, 927–937.
Passari, A. K., Mishra, V. K., Gupta, V. K., Yadav, M. K., Saikia, R., & Singh, B. P. (2015). In vitro and in vivo plant-growth-promoting activities and DNA fingerprinting of antagonistic endophytic actinomycetes associates with medicinal plants. *PLoS ONE*, *10*, e0139468.
Petrolini, B., Quaroni, S., Saracchi, M., & Sardi, P. (1996). Studies on the streptomycete population inhabiting plant roots. *Actinomycetes*, *7*, 66–78.
Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiologiya*, *17*, 362–370.
Ruanpanun, P., Tangchitsomkid, N., Hyde, K. D., & Lumyong, S. (2010). Actinomycetes and fungi isolated from plant-parasitic nematode infested soils: Screening of the effective biocontrol potential, indole-3-acetic acid and siderophore production. *World Journal of Microbiology and Biotechnology*, *26*, 1569–1578.
Sadeghi, A., Karimi, J., Abaszadeh, D., Javid, M. G., Dalvand, Y., & Askari, H. (2012). Plant-growth-promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. *World Journal of Microbiology and Biotechnology*, *28*, 1503–1509.
Saidi, N., Kouki, S., M'hiri, F., Hajlaoui, M. R., Mabrouk, M., Ouzari, H., … Hassen, A. (2009). Characterization and selection of Bacillus sp. strains, effective biocontrol agents against *Fusarium oxysporum* f. sp. *radicis lycopersici*, the causal agent of Fusarium crown and root rot in tomato. *Annals of Microbiology*, *59*, 191–198.
Sardi, P., Saracchi, M., Quaroni, S., Petrolini, B., Borgonovi, G. E., & Merli, S. (1992). Isolation of endophytic *Streptomyces* strains from surface-sterilized roots. *Applied and Environmental Microbiology*, *58*, 2691–2693.
Shimizu, M. (2011). Endophytic actinomycetes: Biocontrol agents and growth promoters. In D. K. Maheshwari (Ed.), *Bacteria in agrobiology: Plant growth responses* (pp. 201–220). Heidelberg: Springer.
Shirling, E. B., & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*, *16*, 313–340.
Singh, P. P., Shin, Y. C., Park, C. S., & Chung, Y. R. (1999). Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria. *Phytopathology*, *89*, 92–99.
Toumatia, O., Compant, S., Yekkour, A., Goudjal, Y., Sabaou, N., Mathieu, F., … Zitouni, A. (2016). Biocontrol and plant growth promoting properties of *Streptomyces mutabilis* strain IA1 isolated from a Saharan soil on wheat seedlings and visualization of its niches of colonization. *South African Journal of Botany*, *105*, 234–239.
Verma, V. C., Singh, S. K., & Prakash, S. (2011). Biocontrol and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica* A. Juss. *Journal of Basic Microbiology*, *51*, 550–556.
Yekkour, A., Sabaou, N., Zitouni, A., Errakhi, R., Mathieu, F., & Lebrihi, A. (2012). Characterization and antagonistic properties of *Streptomyces* strains isolated from Saharan soils, and evaluation of their ability to control seedling blight of barley caused by *Fusarium culmorum*. *Letters in Applied Microbiology*, *55*, 427–435.
Young, W. M., & Crawford, D. L. (1995). Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. *Applied and Environmental Microbiology*, *61*, 3119–3128.
Zamoum, M., Goudjal, Y., Sabaou, N., Barakate, M., Mathieu, F., & Zitouni, A. (2015). Biocontrol capacities and plant growth-promoting traits of endophytic actinobacteria isolated from native plants of Algerian Sahara. *Journal of Plant Diseases and Protection*, *122*, 215–223.