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ORIGINAL ARTICLE

Plant-growth-promoting potential of endosymbiotic actinobacteria isolated from sand truffles (*Terfezia leonis* Tul.) of the Algerian Sahara

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Abstract Seven endosymbiotic actinobacteria were isolated from sand truffles (*Terfezia leonis* Tul.) harvested in the Hassi R'Mel region of the Algerian Sahara. Morphological characteristics and chemotaxonomical analysis indicated that all isolates were members of the *Streptomyces* genus. All the isolated actinobacteria were initially screened in vitro for antifungal capacities, chitinolytic activities, siderophore production, and synthesis of plant-growth regulators (indole-3-acetic acid and gibberellic acid). The isolate *Streptomyces* sp. TL7 exhibited a remarkable profile with positive results in all trials, while the others showed variable responses to assays. In vivo trials were then carried out with the isolates to evaluate their root colonization abilities and plant-growth-promoting potential on tomato (cv. Marmande) seedlings. The results showed that all these *Streptomyces* strains could be isolated successfully from inside the roots of inoculated tomato seedlings. However, the plant-growth-promoting effect varied depending on the treatment. Seeds surface-coated with spores of *Streptomyces* sp. strain TL7 showed the highest performance, with significantly increased ($P<0.05$) shoot and root lengths, and seedling fresh and dry weights. The taxonomic position based on 16S rDNA sequence analysis and phylogenetic studies indicated that strain TL7 was related to *Streptomyces neopeptinius* KNF 2047^T (99.0 % similarity). The interesting antifungal properties and plant-growth promotion traits shown by *Streptomyces* sp. strain TL7 may indicate a potential for its possible use as plant-growth-promoting agent, especially for tomato seedlings.

Keywords *Terfezia leonis* Tul · *Streptomyces* · Antifungal activity · Siderophore · Plant-growth regulators · Plant-growth promotion

Introduction

Since the elaboration of the rhizosphere concept by Lorenz Hiltner in 1904, many studies have reported that the soil environment attached to a root system is a hot-spot of microbial abundance and activity due to the presence of root exudates (Hartmann et al. 2008). Some rhizosphere microorganisms may be neutral or deleterious toward plant growth, whereas other microbes support their hosts (Welbaum et al. 2004).

There is a large body of literature describing the potential uses of plant-associated bacteria, including actinobacteria, as agents to stimulate plant growth and/or manage soil and plant health (Compant et al. 2005; El-Tarabily et al. 2009; Sadeghi et al. 2012). Several actinobacteria of interest have been isolated from the soil, the rhizosphere and also from the roots of various healthy plants (Sabaou et al. 1998; Cao et al. 2004; Sadeghi et al. 2012; Goudjal et al. 2014). Most endophytic actinobacteria remain latent in the internal tissues of non-symptomatic plants without causing any adverse effects in the host (Hasegawa et al. 2006). Nevertheless, other researchers have reported the isolation and description of bacteria (Citterio et al. 1995), yeasts (Buzzini et al. 2005) or

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filamentous fungi (Pacioni et al. 2007) from hypogeous fungi of the *Tuber* genus.

Some endosymbiotic actinobacteria have been demonstrated to improve and promote the growth of host plants, and to exercise an antagonistic, competitive effect on phytopathogenic microbes (Cao et al. 2004; El-Tarabily et al. 2009; Goudjal et al. 2014). They have also been reported for their ability to produce active compounds, such as antifungal and antibacterial compounds or plant-growth regulators (PGRs) that have been developed for agricultural uses (Ilic et al. 2007; Goudjal et al. 2013).

Endosymbiotic actinobacteria assessed in the genus *Streptomyces* are the plant-associated bacteria isolated most frequently from plant tissues (Rosenblueth and Martinez-Romero 2006). Some interesting strains have been used as commercially formulated agents of plant diseases; for example, *Streptomyces griseoviridis* has been used to protect crops against infection by *Fusarium* spp. and *Alternaria* spp. (Lahdenperä et al. 1991).

A number of endosymbiotic actinobacteria inhabit tissues of a wide variety of native and crop plants. Because *in planta* microfloras are so diverse and complicated, associations of endophytic actinobacteria with host plants and/or other endophytes remain poorly understood. Nevertheless, some actinobacteria are beneficial to the life of the host plant and some may play important roles in plant development and health (Shimizu 2011).

Interest in the search for new plant-growth-promoting bacteria (PGPB) has increased considerably over recent years, partly as a response to public concern about the intensive use of chemical pesticides and fertilizers (Fravel 2005). Researchers have turned their attention to different ecological niches, and also to harsh climatic conditions, in the aim of discovering new potential PGPB (Shimizu 2011). In this context, we focused on the study of sand truffles (*Terfezia leonis* Tul.), which have adapted successfully to the stressful conditions of the Algerian Sahara, especially the poor sandy soil and the drought of the arid climate. Sand truffles and their ecological marker plant (*Helianthemum guttatum*) grow and colonize these areas annually without human intervention (Slama et al. 2012). The success of the natural regeneration and the vigorous growth of truffles in the poor sandy soil and arid climatic conditions of the Algerian Sahara might suggest that endosymbiotic actinobacteria help to promote their growth.

The objectives of the present study were to highlight, for the first time, the presence of endosymbiotic actinobacteria that colonize sand truffles (*Terfezia leonis* Tul.) harvested from the Algerian Sahara. The work comprised their preliminary identification, an investigation into their plant-growth-promoting traits, and in vivo assays to evaluate growth promotion in tomato seedlings.

Materials and methods

Sampling of truffles and isolation of endosymbiotic actinobacteria

The choice of sand truffle tubers was based on their abundance and good growth under the harsh conditions of the Sahara. Additionally, no documentation indicates that these tubers have ever been explored previously for actinobacteria isolation. Truffles (*Terfezia leonis* Tul.) were harvested in April 2013 from the region of Hassi R'Mel in the Algerian Sahara (32°56′N, 3°17′E) by the "mark method", in which swollen, cracked soil often reveals the location of the underground truffle. Sand truffles were placed separately in sterile plastic bags and taken to the laboratory for the isolation of actinobacteria.

Tubers were washed in running water to remove sand particles and then sterilized by sequential dipping in 70 % (v/v) ethanol for 5 min, and sodium hypochlorite solution (0.9 % w/v available chlorine) for 20 min. Surface-sterilized tubers were washed three times in sterile distilled water and soaked in 10 % (w/v) $NaHCO_3$ solution for 10 min to disrupt the tissue structure. The epidermis was removed aseptically and the tissue lying beneath was excised in the form of small cubes and placed on chitin-vitamin agar (Hayakawa and Nonomura 1987). Cycloheximide (80 μg mL^{-1}) and nalidixic acid (15 μg mL^{-1}) were added to suppress the growth of fungi and Gram-negative bacteria, respectively. Plates were then incubated at 30 °C for 21 days.

A modification of the method used by Cao et al. (2004) was used to prove that the telluric actinobacteria could not grow after the surface-sterilization protocol. The experiment consisted of soaking the surface-sterilized truffle parts in 100 mL sterile distilled water and shaking at 250 rpm for 20 min. An aliquot of 0.3 mL was then inoculated on chitin-vitamin agar plates, incubated at 30 °C and checked for microbial growth.

Determination of actinobacteria genera

Genera of actinobacteria were determined according to traditional cultural characteristics on yeast extract-malt extract agar (ISP2), oatmeal agar (ISP3) and inorganic salts-starch agar (ISP4) (Shirling and Gottlieb 1966), including the characteristics of colonies, the color of substrates and aerial mycelia, diffusible pigment, and the spore chain morphology (Goodfellow and Simpson 1987). Colors were determined with the ISCC-NBS centroid color charts. The micromorphology and sporulation were observed on the same media by optical microscopy.

The cell wall type was determined on the basis of the diaminopimelic acid (DAP) isomer type to distinguish the *Streptomyces* genus from other spore-forming actinobacteria.

The cell wall hydrolysates of *Streptomyces* strains contained the LL-DAP isomer and other spore-forming actinobacteria contained the DL-DAP isomer (Goodfellow and Simpson 1987).

Antifungal and chitinolytic activities

Antifungal activities of actinobacterial strains against *Fusarium oxysporum* f. sp. *radicis lycopersici*, *Fusarium solani*, *Rhizoctonia solani* AG3, *Umbelopsis ramanniana* NRLL1829 and *Aspergillus carbonarius* M333 from our laboratory collection were determined by the streak assay method (Shirling and Gottlieb 1966). Target fungi were selected on the basis of their pathological effects on tomato seedlings or their widespread occurrence in tomato fields of Algeria (Edel-Hermann et al. 2012). The actinobacterial strain was first cultivated in a straight line on ISP2 (containing 12 g L^{-1} agar) plates, 90 mm in diameter, and incubated for 7 days at 30 °C. After the growth of the strain, the target fungi were seeded in streaks perpendicular to those of actinobacteria cultivation and the antifungal activity was evaluated by measuring the distance of inhibition between target fungal and actinobacterial colony margins, after incubation at 25 °C for 5 days.

Chitinolytic activities were assessed using colloidal chitin medium (CC) containing chitin as the sole carbon source (Wang et al. 2013). Clear-zone diameters were measured and used to indicate the positive chitinolytic activity of each isolate.

Siderophore production

Siderophore production was screened on chrome azurol S (CAS) plates (Sadeghi et al. 2012). Plugs 6 mm in diameter taken from actinobacteria cultures on ISP2 medium were placed on these plates and incubated at 30 °C for 7 days. Colonies producing an orange or red zone were considered as positive for siderophore production.

Plant-growth regulator production

All actinobacteria were screened for their indol-3-acetic acid (IAA) and gibberellic acid (GA3) production abilities using the methods of Tien et al. (1979) and Khamna et al. (2010). Aliquots of 1 mL of spore suspension of actinobacterial strains (10^6 CFU mL^{-1}) were transferred into 250-mL Erlenmeyer flasks containing 50 mL yeast extract-tryptone broth supplemented with 5 mg mL^{-1} L-tryptophan (Khamna et al. 2010). The same quantity of ISP2 broth was used for GA3 production. Flasks were cultured on a rotary shaker (200 rpm) at 30 °C for 5 days and supernatant cultures were harvested by centrifugation at 10,000 rpm for 30 min.

IAA production was revealed by mixing 2 mL supernatant culture with 4 mL Salkowski reagent. The appearance of a pink color after 30 min in a dark room indicated positive IAA production. Optical density was read at 530 nm using a spectrophotometer (JANWAY-6405; http://www.jenway.com) and the level of IAA produced was determined from a standard IAA graph (Acros Organics) (Ruanpanun et al. 2010).

For GA3 investigation, after the supernatant culture was recovered the cell pellet was re-extracted with phosphate buffer (pH 8.0) and centrifuged again. Both supernatants were pooled, acidified to pH 2.5 by the addition of 5 *N* hydrochloric acid and partitioned with equal volumes of ethyl acetate five times. Zinc acetate solution (2 mL; 10 % w/v) and 2 mL potassium ferrocyanide (1 % w/v) were added to 15 mL ethyl acetate fractions and the mixture centrifuged at 10,000 rpm for 10 min. Five milliliters of supernatant was added to 5 mL hydrochloric acid (30 % v/v) and the mixture was incubated at 20 °C for 75 min. The absorbance was measured at 254 nm and the amount of GA3 produced was calculated from a standard graph using standard GA3 (Sigma-Aldrich, St. Louis, MO) solution.

Thin layer chromatography (TLC) was used to identify the PGRs. TLC chromatograms were run on silica gel (GF254, thickness 0.25 mm, Merck, Darmstadt, Germany) and the solvent system was chloroform : ethyl acetate : formic acid (50:40:10, v:v:v) to separate IAA and GA3 into ethyl acetate fractions. IAA and other indolic compounds were detected on TLC plates by spraying with Ehmann's reagent. Gibberellins were revealed by spraying the chromatograms with ethanol : sulfuric acid (90:10, v:v) and heating to induce fluorescence of the compounds in ultraviolet light (254 nm). Spots of IAA and GA3 produced were identified by Rf values that were identical to authentic PGRs (Tien et al. 1979; Ruanpanun et al. 2010).

Internal colonization of tomato seedling roots

All actinobacteria isolates were tested for their ability to colonize the root interior of tomato (cv. Marmande) seedlings according to the method used by El-Tarabily et al. (2009) and Shi et al. (2009). Surface-sterilized tomato seeds were germinated in autoclaved sandy soil for 10 days. The root tips (about 3 mm) were trimmed using a sterile scalpel to facilitate uptake of the bacterial inoculum. Seedlings were then placed, separately, in sterile glass tubes at 25 °C for 24 h with only their roots submerged in the inoculum suspension (10^6 CFU mL^{-1}) of each isolate. Control seedlings with severed root tips were treated with autoclaved inoculum. The inoculated seedlings were then planted in sterile sandy soil pots under greenhouse conditions. After 30 days, the roots were carefully removed from the soil and any endosymbiotic actinobacteria were isolated using the protocol described previously.

In vivo growth promotion of tomato seedlings

Microbial suspensions and coating of tomato seeds

Actinobacteria suspensions were prepared according to Errakhi et al. (2007) by cultivating the actinobacteria isolates on ISP2 agar at 30 °C for 10 days. Tween-80 solution (0.05 %) was used to cover spores that had been adjusted to 10^6 CFU mL^{-1} using the Thoma cell.

Coating of tomato seeds was carried out as described by Goudjal et al. (2013). Seeds were surface-sterilized by dipping in 70 % (v/v) ethanol for 5 min followed by sodium hypochlorite solution (0.9 % w/v available chlorine) for 4 min. The seeds were then washed three times in sterile distilled water. Sterilized seeds were coated separately by soaking for 30 min in the actinobacterial suspensions and then dried under a laminar flow hood. Coated seeds were stored at 6 °C for no longer than 1 day before sowing.

In vivo plant-growth-promoting trials

All actinobacterial isolates were screened in vivo for their PGPB effects on tomato (cv. Marmande) seedlings. Cultures were grown in autoclaved compost (Jardiland, France; http://www.jardiland.com/) conditioned in plastic pots (10 cm high x 8 cm in diameter). The control culture consisted of sowing surface-sterilized seeds without coating treatment. However, coated tomato seeds were used to evaluate the effect of each actinobacterial strain on the promotion of seedling growth.

For each treatment, six tomato seeds were sown per pot at 5 mm below the soil surface, with ten replicates per treatment using a fully randomized complete block design. The tomato cultures were grown under greenhouse conditions (25 °C, 14 h light/10 h dark). Pots were watered daily with 10 mL tap water to maintain a moisture level favourable for seed germination.

After 6 weeks, seedlings were carefully removed from the soil and washed with tap water. The shoot and root lengths, and the fresh and dry weights were measured.

Molecular taxonomy of *Streptomyces* sp. TL7

The identity of the isolate (TL7) with the highest potential plant-growth promoting activity for tomato cv. Marmande seedlings was confirmed by 16S rRNA gene sequence analysis. Genomic DNA was prepared according to the CTAB method (Liu et al. 2000). The 16S rRNA gene sequence was amplified by the PCR method with *Taq* DNA polymerase and primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′). The 16S rRNA gene sequence was amplified by PCR using a lit from Invitrogen (Carlsbad, CA). The final 50 μL volume of reaction mixture contained 1X PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 9.0 at 25 °C), 1.5 mM $MgCl_2$, 200 μM each dNTP, 1 μM each primer, 1.25 U *Taq* DNA polymerase and 1 μL (500 ng) purified DNA. The amplification was performed on a thermal cycler (RoboCycler Gradient 96, Stratagene, LaJolla, CA) according to the following profile: an initial denaturation step at 98 °C for 3 min, after which *Taq* polymerase was added, followed by 30 amplification cycles of 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min, and a final extension step of 72 °C for 10 min. The PCR product was detected by agarose gel electrophoresis and was visualized by ultraviolet fluorescence after ethidium bromide staining. The PCR products obtained were sent to the Beckman Coulter Genomics Company (Tekeley, UK) for sequence determination. The16S rRNA sequence has been deposited with the GenBank data library and assigned the accession number KM891590. The sequence obtained was compared with sequences present in the public sequence databases and with EzTaxon tools (Chun et al. 2000).

Statistical analysis

Three replications were carried out for each experiment and values represent the mean±standard deviation. Data were subjected to analysis of variance (ANOVA) and significant differences between means were compared using Fisher's protected LSD test at $P=0.05$. Differences were considered significant when $P<0.05$.

Results

Taxonomical characteristics of endosymbiotic actinobacteria

Seven endosymbiotic actinobacteria were isolated and purified from sand truffles of the Algerian Sahara. Morphological analysis showed that all isolates produced aerial mycelia and non-fragmented substrate on ISP2, ISP3 and ISP4 media. Based on their aerial mycelium and spore-mass color, actinobacterial isolates were classified into grey (4 isolates) or red (3 isolates) color groups. Microscopic analysis showed that all isolates had the spore-chain type *Spira* (S), with approximately 10–50 spores per chain. The chemotaxonomic analysis carried out on the cell wall hydrolysates revealed the presence of LL-DAP isomer for all actinobacterial isolates. Based on these taxonomic characteristics, the seven isolates were assigned to the genus *Streptomyces* (Table 1).

Samples of the water used to wash surface-sterilized truffles sown on chitin-vitamin agar failed to grow any microbial colonies. This proves the efficacy of the treatment process to eradicate telluric microbes from the surface of truffles. Consequently, all the actinobacteria isolated were localized in the interior of truffle tubers.

Table 1 Morphological characteristics and diaminopimelic acid isomer of the *Streptomyces* spp. isolates. *S* Spira, *DAP* diaminopimelic acid

Isolate	Color of			Spore-chain type	Color group	DAP isomer	Genus
	Substrate mycelium	Aerial mycelium	Diffusible pigment				
TL1	Dark brown	Pink	Medium Brown	S	Red	LL	*Streptomyces*
TL2	Medium brown	Pinkish white	/	S	Red	LL	*Streptomyces*
TL3	Light brown	Grey	/	S	Grey	LL	*Streptomyces*
TL4	Light brownish grey	Grey	Brown	S	Grey	LL	*Streptomyces*
TL5	Light brownish grey	Grey	/	S	Grey	LL	*Streptomyces*
TL6	Light yellowish brown	Pinkish white	Yellowish brown	S	Red	LL	*Streptomyces*
TL7	Brownish grey	Grey	Brown	S	Grey	LL	*Streptomyces*

Antifungal and chitinolytic activities

The *Streptomyces* isolates showed apparent antifungal activity towards all phytopathogenic fungi tested. Results were expressed by the distance (inhibition zone) between the margins of the target fungal and actinobacterial colonies (Table 2). Among all *Streptomyces* isolates, the maxima of antifungal activities were obtained with *Streptomyces* sp. TL7, which inhibited the mycelium growth of all targeted fungi. TL7 also showed a larger inhibition zone than the other *Streptomyces* strains. Furthermore, all the *Streptomyces* isolates showed antifungal activities against *Fusarium solani*, while *Rhizoctonia solani* AG3 was inhibited by only two strains of *Streptomyces*.

Chitinolytic activity was measured by the diameter of the clear halo around the *Streptomyces* colonies on CC plates (Table 2). All isolates of *Streptomyces* spp. were able to degrade colloidal chitin. Nevertheless, the highest chitinolytic activity was reached by strain *Streptomyces* sp. TL7.

Siderophore and plant-growth regulator production

Results for siderophore production are shown in Table 3. Screening for siderophore production showed that three *Streptomyces* isolates (TL4, TL6 and TL7) were found to produce a clear zone on CAS medium. Therefore, they qualified as siderophore-producing strains.

Results for IAA and GA3 production by the *Streptomyces* isolates are given in Table 3. All the isolates except *Streptomyces* sp. TL6 were able to produce IAA in the range of 35.9–117.1 μg mL^{-1}, and *Streptomyces* sp. TL7 achieved maximum IAA production. However, GA3 production was observed only with *Streptomyces* spp. TL4 and TL7, which produced the highest amount (42.7 μg mL^{-1}).

Internal root colonization ability

Results for the root colonization abilities of the *Streptomyces* isolates showed no visible growth of endosymbiotic actinobacteria from the control seedlings. However, all strains were isolated successfully from the surface-sterilized roots of inoculated tomato seedlings. This confirmed the endosymbiotic origin of the isolates from the sand truffles, and demonstrated their ability to colonize the roots of tomato seedlings.

Identification of *Streptomyces* sp. TL7

Molecular taxonomy and phylogenetic analysis using the 16S rDNA sequence (GenBank KM891590) for the strain TL7,

Table 2 Antifungal and chitinolytic activities of the *Streptomyces* spp. isolates

Isolate	Distance of inhibition zone (mm) [a]					Chitinolytic activity [a] clear zone diameter (mm)
	Fusarium oxysporum f. sp. *radicis lycopersici*	*Fusarium solani*	*Rhizoctonia solani* AG3	*Umbelopsis ramanniana* NRLL 1829	*Aspergillus carbonarius* M333	
TL1	00	06±0.9	10±1.5	00	00	12±0.6
TL2	12±0.8	18±1.2	00	12±1.0	00	16±0.4
TL3	00	12±1.2	00	06±0.6	10±0.6	16±0.8
TL4	03±0.6	07±0.6	00	00	10±1.6	14±0.4
TL5	06±0.6	08±0.9	00	14±1.3	00	10±0.6
TL6	00	15±1.6	00	00	05±1.0	12±0. 4
TL7	18±1.2	20±1.0	12±0.9	16±1.2	18±1.2	18±0.9

[a] Average±standard deviation (SD) from three replicates

Table 3 Siderophore, indole-3-acetic acid (IAA) and gibberellic acid (GA3) production by *Streptomyces* spp. isolates. *PGRs* Plant-growth regulators, *CAS* chrome azurol S

Isolate	Siderophore production	PGR production [a]	
	Halo diameter on CAS medium (mm)[a]	IAA (μg mL^{-1})	GA3 (μg mL^{-1})
TL1	00	44.4±1.7	00
TL2	00	37.9±0.4	00
TL3	00	84.1±0. 6	00
TL4	26±1.3	81.1±1.2	42.7±0.9
TL5	00	35.9±0.5	00
TL6	08±0.8	00	00
TL7	22±1.3	117.1±0.6	55.3±0.7

[a] Average±SD from three replicates

which showed the highest plant-growth-promoting potential on tomato seedlings, confirmed that this strain belonged to the genus *Streptomyces*. The similarity level with *Streptomyces neopeptinius* KNF 2047^T—the most closely related species—was 99.0 %.

In vivo plant-growth-promoting activities

The PGPB activities of *Streptomyces* spp. isolates on tomato seedlings are shown in Fig. 1. Different results for the shoot and root lengths and the seedling fresh and dry weights were obtained for each treatment. Tomato seed treatments with spores of *Streptomyces* spp. TL1, TL2, TL3, TL4, TL5 and TL6 were unable to promote the growth of tomato seedlings significantly considering the evaluated parameters. However, significant differences were found between the results of the control culture and those obtained from seeds coated with spores of isolate TL7 (Fig. 1).

Shoot length (11.5 cm), root length (7.3 cm) and fresh (5.1 g) and dry (2.0 g) weights of the control culture were increased significantly by *Streptomyces* sp. TL7, which showed the highest PGPB effect. This strain enhanced the shoot length to 16.1 cm, the root length to 10.9 cm, the fresh weight to 8.0 g and the dry weight to 3.2 g.

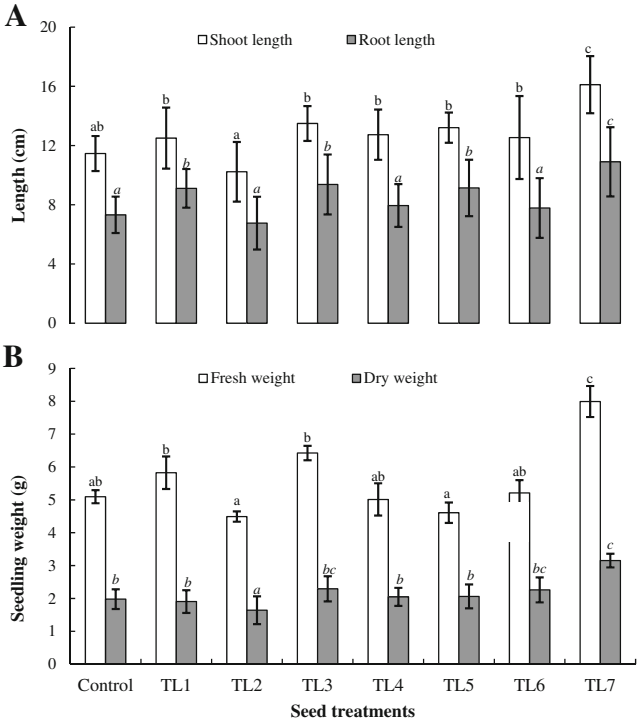

Fig. 1 Effect on **a** the shoot and root lengths and **b** seedling fresh and dry weights of coating tomato (cv. Marmande) seeds with spores of *Streptomyces* spp. TL1, TL2, TL3, TL4, TL5, TL6 and TL7 isolates. Evaluation was made 6 weeks after planting. *Bars* labelled with the same *letters* are not significantly different according to Fisher's protected LSD test at $P=0.05$. *Error bars* Standard deviation (SD) from ten replicates

Discussion

The present study described seven strains of actinobacteria isolated from the inside of sand truffles of the Algerian Sahara. To eliminate telluric microbes, tuber truffles were surface-sterilized and all strains proved to be associated with truffle interior tissues and were isolated from inside the tubers.

A large body of literature reports the isolation of actinobacteria from the soil, rhizosphere and phyllosphere (Hartmann et al. 2008; Sadeghi et al. 2012). Actinobacteria are a group of bacteria that are distributed widely in the Saharan soils of Algeria (Sabaou et al. 1998). They can make important contributions to nutrient cycling of natural substrates and ultimately affect soil nutrition (Shimizu 2011) and the growth of native Saharan plants (Goudjal et al. 2014). In the rhizosphere, root exudates selectively influence the growth of microorganisms that colonize the rhizosphere. Metabolites from highly adapted root-colonizing actinobacteria are, in turn, an important source of the antibiotics, enzymes, and PGRs involved in promoting plant growth and preventing the invasion of roots by some soil-borne pathogenic fungi (Tokala et al. 2002; Shimizu 2011).

In the search for efficient PGPB strains with multiple activities, endophytic actinobacteria have been isolated widely from roots, stems and leaves of crop, woody and medicinal plants (Shimizu 2011). However, some studies demonstrate that only hypogeous fungi, such as *Tuber* spp., host various microorganisms. These fungi contain a great number of bacteria (Citterio et al. 1995) and several yeasts (Buzzini et al. 2005) in their gleba. Additionally, several workers have reported the isolation of filamentous fungi such as hyphomycetes from healthy ascomata of various truffle species (Pacioni et al. 2007). However, the literature reports no research on

endosymbiotic microbes from sand truffles, and this is the first description of the isolation of actinobacteria from truffles of the Algerian Sahara.

A number of endophytic actinobacteria inhabit the rhizosphere and tissues of a wide variety of plants native to the Sahara (Goudjal et al. 2013). Some of them are undoubtedly beneficial to the life of the host plant. They colonize the host's internal tissues, usually obtaining nutrition and protection from the host organism. In return, they confer enhanced fitness on the host plants by producing certain functional metabolites (Tan and Zou 2001). Shimizu (2011) suggests that the endophytic presence of some actinobacteria may play an important role in the development and health of hosts. They probably produce antimicrobial metabolites within their host plants (Tan and Zou 2001) and PGRs that enhance the growth of their hosts (El-Tarabily et al. 2009). They have been demonstrated to improve and promote the growth of host plants, as well as producing PGRs and reducing disease symptoms (El-Tarabily et al. 2009; Goudjal et al. 2013). Furthermore, actinobacteria can play a role in nutrient assimilation, such as the solubilization of mineral phosphates and other nutrients (Hamdali et al. 2008).

On the basis of the morphological characteristics and the cell wall type, all endosymbiotic actinobacteria isolated from sand truffles were assigned to the *Streptomyces* genus. Thakur et al. (2007) reported that the actinobacteria most frequently found in the soil were of the *Streptomyces* genus. This is the largest and most dominant genus in the sand soil of the Algerian Sahara (Sabaou et al. 1998). Likewise, *Streptomyces* species are the species most frequently isolated from plant tissues, suggesting that these species could have high compatibility with a wide range of host organisms and contribute to improvement of their growth (Rosenblueth and Martinez-Romero 2006; Verma et al. 2009; Goudjal et al. 2014).

Actinobacteria are well known for their capacity to produce bioactive compounds. Most of these microorganisms have been isolated from soil samples. Therefore, researchers are now investigating less well-explored ecological niches, searching for different microorganisms that could produce new compounds (Fialho de Oliveira et al. 2010). Endosymbiotic actinobacteria are a promising source of new bioactive compounds that have been isolated from several plant species (El-Tarabily et al. 2009).

The seven endosymbiotic actinobacteria isolated in our study showed antifungal activity against at least one of the target fungi, and the strain *Streptomyces* sp. TL7 showed the strongest antagonistic activity. Antifungal activities of endosymbiotic actinobacteria observed in this study are in accordance with previous reports (Errakhi et al. 2007; Ilic et al. 2007). In addition, several studies have reported the antagonistic activities of actinobacteria isolates toward a variety of plant pathogenic fungi, such as *Aspergillus* (Valois et al. 1996), *Pythium* (Hamdali et al. 2008), *Fusarium* (Yekkour et al. 2012) and *Rhizoctonia* (Goudjal et al. 2014).

The physical destruction of the fungal cell wall by the action of extracellular hydrolytic enzymes produced by actinobacteria is an important mechanism of antagonism. Production of chitinases by antagonistic actinobacteria is often involved in biological control processes (Gonzalez-Franco et al. 2003). In this study, all endosymbiotic actinobacteria showed chitinolytic activities on CC medium. Beside the production of antifungal compounds, chitinolytic activity can be implicated in antagonistic activities. These findings are in agreement with those of Hoster et al. (2005), who reported the role of chitinase-producing *Streptomyces* in antifungal activity against some phytopathogenic fungi.

Siderophores are low-molecular-weight compounds secreted by many bacteria that permit the acquisition of ferric ion (Whipps 2001). Due to the importance of iron for growth and metabolism, siderophore-mediated iron acquisition plays an important role in the ability of a microorganism to colonize plant roots (Crowley 2006).

Among all the actinobacteria tested, only three isolates were found to be able to produce siderophores on CAS medium, where a clear zone appeared around colonies. These results are in agreement with those reported by Cao et al. (2005), who specified the role of siderophore-producing endophytic *Streptomyces* sp. S96 in the antagonistic effect on *Fusarium oxysporum* f. sp. *cubens*. Several studies have reported the role of *Streptomyces* spp. in the production of diverse siderophores having high affinity for ferric iron, thereby sequestering iron away from pathogens and restricting their growth (Whipps 2001; Tokala et al. 2002).

Tokala et al. (2002) reported that the PGPB colonize the rhizosphere of numerous plant species and confer diverse beneficial effects. Their activities occur when a PGPB facilitates uptake of soil nutrients by the plant or when the plant is otherwise supplied with a compound that is synthesized by the symbiotic bacteria, such PGRs (Lynch and Whipps 1990).

A significant number of endosymbiotic actinobacteria are able to produce PGRs such as auxins, gibberellins and cytokinins, which significantly increase root and shoot length (El-Tarabily et al. 2009; Merzaeva and Shirokikh 2010; Goudjal et al. 2013).

With the exception of *Streptomyces* sp. TL6, all endosymbiotic actinobacteria produced at least one of the two PGRs studied. Compared to the other endosymbiotic isolates, it should be noted that *Streptomyces* sp. TL7 synthesized the largest quantities of IAA and GA3. Our findings are in agreement with several papers reporting that endophytic actinobacteria isolated from various plants are able to produce PGRs, especially IAA and GA3 (Tokala et al. 2002; El-Tarabily et al. 2009; Goudjal et al. 2013). Furthermore, Bottini et al. (2004) and El-Tarabily et al. (2009) reported that gibberellins are synthesized by a number of bacteria and are

also involved in a number of developmental processes in plants.

The ability of all the *Streptomyces* isolates to colonize tomato roots demonstrates that their endosymbiotic properties are maintained. Despite their unusual native biotope, *Streptomyces* isolates are able to colonize the roots of tomato seedlings successfully. As defined by Wilson (1995), endosymbiotic actinobacteria, for all or part of their life cycle, invade the tissue of living plants and cause unapparent and asymptomatic infections entirely within plant tissue, but cause no symptoms of disease. Similar results were obtained by El-Tarabily et al. (2009), highlighting the possibility of internal colonization of tomato and cucumber roots by various *Streptomyces* strains. Re-isolation of actinobacteria from inoculated tomato seedlings can be explained by their effective colonization of living root cells as reported by El-Tarabily et al. (2009), who found *Actinoplanes campanulatus* sporangia within cells of healthy cucumber roots. Nevertheless, re-isolation could be explained by the passive penetration of bacteria into severed xylem vessels, their distribution through the plant tissues and their possible colonization of intercellular spaces as revealed by Muzammil et al. (2013), who reported that *Saccharothrix algeriensis* NRRL B-24137 colonized spaces between rhizodermal root cells of *Arabidopsis thaliana*.

A dependence of plant growth enhancement on the inoculation of *Streptomyces* under gnotobiotic conditions was reported by Hamdali et al. (2008), Shimizu (2011) and Sadeghi et al. (2012). Furthermore, Meguro et al. (2006) reported an endophytic *Streptomyces* sp., strain MBR-52, that accelerated the emergence and elongation of plant adventitious roots. The effect on seedling growth of coating tomato seeds with spores of our endosymbiotic *Streptomyces* isolates is highlighted in Fig. 1. In these results, *Streptomyces* sp. TL7 seems to be correlated with root enhancement and shoot production. These PGPB effects can be explained, as in the interpretation by Shi et al. (2009), by the actions of auxins and gibberellins produced by *Streptomyces* sp. TL7 on root and stem elongations. Our findings agree with the results of El-Tarabily et al. (2009), who reported a significant enhancement of cucumber plant growth by endophytic actinobacteria producing IAA. In addition, several endophytic actinobacteria have been reported to produce PGRs in vitro and to promote the growth of seedlings (El-Tarabily et al. 2009; Goudjal et al. 2013). Plant-growth promotion can also be attributed to other factors, such as siderophore production and inorganic phosphate solubilization (Hamdali et al. 2008). El-Tarabily et al. (2009) reported that the involvement of PGRs could not only help seedlings to grow better but could also help the host to compensate for tissue damage caused by the pathogenic agent.

Strain TL7 is closely related to *S. neopeptinius*. Several studies have reported the role of rhizospheric and endophytic *Streptomyces* strains as potential agents in the promotion of plant growth (Cao et al. 2004; Meguro et al. 2006; El-Tarabily et al. 2009; Sadeghi et al. 2012; Goudjal et al. 2013). However, this is the first report showing the role of *S. neopeptinius* as a potential plant-growth-promoting actinobacteria. Furthermore, *S. neopeptinius* have never been reported for its properties as a potential plant-growth-promoting actinobacteria.

Our study is the first to show the isolation of endosymbiotic *Streptomyces* strains from desert truffles (*Terfezia leonis* Tul.) harvested in the Algerian Sahara, their in vitro characterization and their in vivo implication in the improvement of plant-growth in tomato seedlings. Despite its unusual native biotope, strain *Streptomyces* sp. TL7 showed interesting antagonistic activities and plant-growth promotion effects on tomato (cv. Marmande) seedlings. These properties open up promising perspectives for its application in crop improvement. In this case, it would be interesting to characterize isolate *Streptomyces* TL7 to investigate its mycorrhization properties, its improvement of the uptake of minerals such as solubilization of inorganic phosphate, the effectiveness of its in vivo biocontrol of soil-borne pathogenic fungi, and to carry out formulation assays of its spores.

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