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Endophytic actinomycetes from spontaneous plants of Algerian Sahara: indole-3-acetic acid production and tomato plants growth promoting activity

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Abstract Twenty-seven endophytic actinomycete strains were isolated from five spontaneous plants well adapted to the poor sandy soil and arid climatic conditions of the Algerian Sahara. Morphological and chemotaxonomical analysis indicated that twenty-two isolates belonged to the *Streptomyces* genus and the remaining five were non-*Streptomyces*. All endophytic strains were screened for their ability to produce indole-3-acetic acid (IAA) in vitro on a chemically defined medium. Eighteen strains were able to produce IAA and the maximum production occurred with the *Streptomyces* sp. PT2 strain. The IAA produced was further extracted, partially purified and confirmed by thin layer chromatography (TLC) analysis. The 16S rDNA sequence analysis and phylogenetic studies indicated that strain PT2 was closely related to *Streptomyces enissocaecilis* NRRL B 16365^T, *Streptomyces rochei* NBRC 12908^T and *Streptomyces plicatus* NBRC 13071^T, with 99.52 % similarity. The production of IAA was affected by cultural conditions such as temperature, pH, incubation period and L-tryptophan concentration. The highest level of IAA production (127 µg/ml) was obtained by cultivating the *Streptomyces* sp. PT2 strain in yeast extract-tryptone broth supplemented with 5 mg L-tryptophan/ml at pH 7 and incubated on a rotary shaker (200 rpm) at 30 °C for 5 days. Twenty-four-hour treatment of tomato *cv*. Marmande seeds with the supernatant culture of *Streptomyces* sp. PT2 that contained the crude IAA showed the maximum effect in promoting seed germination and root elongation.

Keywords Endophytic actinomycetes · Indole-3-acetic acid · Plant growth promotion · *Streptomyces* · Tomato *cv*. Marmande

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Introduction

Endophytes are defined as "bacteria or fungi that, for all or part of their life cycle, invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissue but cause no symptoms of disease" (Wilson 1995). The first identified endophytic actinomycete capable of fixing molecular nitrogen belonged to the genus *Frankia* (Benson and Silvester 1993). In the last decades, other endophytic actinomycetes, such as *Streptomyces*, *Nocardia*, *Amycolatopsis*, *Micromonospora* and *Microbispora*, have been isolated from surface-sterilized roots of various plant species (Coa et al. 2005; Shi et al. 2009; Ruanpanun et al. 2010). Actinomycetes are widely represented in soils and play various beneficial roles. They occupy the plant rhizosphere soil and produce active compounds, such as antifungal and bactericidal compounds, or plant-growth-promoting substances that have been commercialized for agricultural uses (Suzuki et al. 2000; Ilic et al. 2007). For example, some endophytic

actinomycetes have positive effects on host plants by producing plant growth regulators (Ting et al. 2008). They can promote plant growth by producing phytohormones such as auxins or gibberellins (Kaunat 1969; Brown 1972; Merckx et al. 1987). The auxins are a group of indole ring compounds that have the ability to improve plant growth by stimulating seed germination, root initiation, cell elongation, and seedling growth (El-Tarabily et al. 2008). Indole-3-acetic acid (IAA) is a common natural auxin and is a product of L-tryptophan metabolism in microorganisms (Sapak et al. 2008; Ruanpanun et al. 2010). Several *Streptomyces* species, such as *S. olivaceoviridis*, *S. rimosus* and *S. viridis*, have the ability to produce IAA and improve plant growth by increasing seed germination, root elongation and root dry weight (Aldesuquy et al. 1998; Tokala et al. 2002; El-Tarabily et al. 2008; Khamna et al. 2010).

In the Algerian Sahara, several plant species have successfully adapted to the stressful conditions of the region, especially the poor sandy soil and the drought of the arid climate. Spontaneous plants grow and colonize these areas without human intervention. They contribute to dune fixation and can also be explored for pastoral and clinical uses.

The aim of the present work is to bring out the effect of endophytic actinomycetes in IAA production for an agronomic application. The vigorous growth of spontaneous plants under the harsh conditions of the Algerian Sahara could suggest a contribution of endophytic microbes producing plant growth regulators, such as auxins, to the promotion of plant growth. For the purposes of the study, endophytic actinomycetes were isolated and screened in vitro for their abilities to produce IAA on a defined chemical medium. Culture conditions were optimized for the strain producing the most IAA. The effects of the IAA produced on seed germination and root elongation of tomato (*Solanum lycopersicum*) *cv*. Marmande were studied.

Materials and methods

Sample collection

Five different species of spontaneous herbaceous plants (*Cleome arabica*, *Solanum nigrum*, *Astragallus armatus*, *Aristida pungens* and *Panicum turgidum*) were collected from the Hassi R'mel region of the Algerian Sahara (32°56′N, 3°17′E) in March 2011. Plant species for endophyte isolation were selected on the basis of their abundance and ethnobotanical properties, such as therapeutic uses, pastoral properties and dune fixing abilities. No records indicate that these plants have been studied for endophytic actinomycetes isolation previously. Five healthy root samples of each plant were placed in sterile plastic bags, taken to the laboratory, and processed immediately.

Isolation of endophytic actinomycetes

Endophytic actinomycete strains were isolated according to the method used by Taechowisan et al. (2003) and Coa et al. (2004). Representative root samples (2–5 mm in diameter) from each plant species were washed in tap water to remove soil particles and then sterilized by sequential immersion in 70 % (v/v) ethanol for 5 min and sodium hypochlorite solution (0.9 % w/v available chlorine) for 20 min. The surface-sterilized root samples were then washed by being immersed in sterile distilled water three times to remove the surface sterilization agents. Next, the root samples were divided into thin discs (0.2 × 0.5 cm) and placed on chitin-vitamin B agar (Hayakawa and Nonomura 1987). Cycloheximid (80 mg/l) and nalidixic acid (15 mg/l) were added to suppress the growth of fungi and Gram-negative bacteria respectively. The plates were then incubated at 30 °C for 21 days.

Validation of the surface sterilization protocol

Two experimental methods were used to validate the surface sterilization protocol. The first consisted of submerging endophytic actinomycetes colonies grown on chitin-vitamins B agar in 70 % (v/v) ethanol for 5 min and sodium hypochlorite solution (0.9 % w/v available chlorine) for 20 min. Submerged actinomycete cultures were then incubated on the same medium and the viability of actinomycetes was recorded. The second method consisted of soaking the surface-sterilized root samples in 5 ml sterile distilled water and stirring for 1 min. An aliquot of 0.3 ml suspension was then inoculated on chitin-vitamins B agar, incubated at 30 °C, and checked for microbial growth (Coa et al. 2004; Tan et al. 2006).

Preliminary identification of endophytic actinomycetes

Actinomycete strains belonging to the *Streptomyces* genus were identified 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). Chemical analysis of the cell components was carried out by Becker et al. (1964) method for the determination of the isomeric form of diaminopimelic acid (DAP). Whole-organism hydrolysates of *Streptomyces* strains contain the LL isomer and other spore-forming actinomycetes contain *meso*-diaminopimelic acid (Goodfellow and Simpson 1987).

Molecular identification of *Streptomyces* sp. PT2

Identification of the strain with the highest level of IAA production (*Streptomyces* sp. PT2) was confirmed by the 16S rDNA gene sequence analysis. Genomic DNA was prepared according to the CTAB method (Liu et al. 2000). The 16S rDNA was amplified using two primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GG TTACCTTGTTACGACTT-3′). The 16S rRNA gene sequence was amplified by PCR using an Invitrogen kit. The final 50 µl volume of reaction mixture contained 1X PCR buffer (10 mM of Tris–HCl, 50 mM of KCl, pH 9.0 at 25 °C), 1.5 mM of $MgCl_2$, 200 µM of each dNTP, 1 µM of each primer, 1.25 U of Taq DNA polymerase and 1 µl (500 ng) of purified DNA. The amplification was performed on a thermal cycler (STRATAGENE RoboCycler Gradient 96) 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 (UV) fluorescence after ethidium bromide staining. The PCR products obtained were sent to the MilleGen Company (Toulouse, France) for sequence determination. The same primers as above and an automated sequencer were used for this purpose. The 16S rRNA sequence has been deposited in the GenBank data library and assigned the accession number KC414013. The sequence obtained was compared with sequences present in the public sequence databases and with EzTaxon, a web-based tool for the identification of prokaryotes based on 16S rRNA gene sequences from type strains (Chun et al. 2000). The phylogenetic tree was constructed using the Molecular Evolution Genetics Analysis (MEGA) software version 5.0 (Tamura et al. 2011). The 16S rRNA sequence of strain PT2 was aligned using CLUSTAL W program (Larkin et al. 2007) against corresponding nucleotide sequences retrieved from GenBank and the phylogenetic tree was constructed using the neighbour-joining method (Saitou and Nei. 1987) with the Kimura 2-parameter model (Kimura 1980). The topology of the tree was evaluated by bootstrap analysis based on 1,000 replicates (Felsenstein 1985).

Indole-3-acetic acid (IAA) production

All endophytic actinomycetes were screened for their IAA production abilities according to the methods used by Ahmad et al. (2005); Ruanpanun et al. (2010); Vikram (2011) and Sadeghi et al. (2012). One-millilitre aliquots of the spore suspensions of the actinomycete strains ($\approx 10^6$ c.f.u/ml) were transferred into 500 ml-Erlenmeyer flasks containing 100 ml of yeast extract–tryptone broth (YT) (5 g yeast extract/l, 10 g tryptone/l, 5 g NaCl/l, pH 7.2) supplemented with 5 mg/ml of L-tryptophan. Flasks were cultured on a rotary shaker (200 rpm) at 30 °C for 5 days and supernatant cultures were harvested by centrifugation at 4,000 rpm for 30 min. The IAA production was revealed by mixing 2 ml of the supernatant culture with 4 ml of Salkowski reagent (1 ml of 0.5 M $FeCl_3$ in 49 ml of 35 % (w/v) $HClO_4$). The appearance of a pink color after 30 min in a dark room indicated that IAA production had occurred. Optical density was read at 530 nm using a spectrophotometer (JANWAY-6405) and the IAA concentration was estimated using a pure IAA standard graph.

Extraction and detection of IAA

The supernatant culture that showed the highest IAA concentration was used for IAA extraction according to the method described by Ahmad et al. (2005). The supernatant was acidified to pH 2.5 with HCl and the IAA was extracted using a supernatant culture:ethyl acetate ratio of 1:2 by vol.

The IAA production was confirmed by thin layer chromatography (TLC). Ethyl acetate fractions (10–20 µl) 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 (254 nm) after spraying the plates with Ehmann's reagent (Xie et al. 1996; Ahmad et al. 2005; Vikram 2011).

Screening for optimal conditions for IAA production

To optimize culture conditions for IAA production by *Streptomyces* sp. PT2, the effects of incubation period, temperature, pH, and L-tryptophan concentration on bacterial growth and IAA production were studied. IAA concentration was measured by fixing all culture parameters and then changing one parameter at a time. The same medium, YT, and culture conditions as described above (IAA production) were used. The effect of incubation period on IAA production was studied for 10 days, and the effects of temperature (20, 25, 30, 35 and 40 °C), pH (4, 5, 6, 7, 8, 9 and 10) and the concentration of L-tryptophan (0, 1, 2, 3, 4, 5, 6 and 7 mg/ml) were studied for 7 days.

Plant growth promoting activity of the IAA produced

In order to examine the seed germination and root elongation effects of IAA produced by the strain *Streptomyces* sp. PT2 on tomato *cv*. Marmande (the variety most cultivated in greenhouses in Algeria), three treatments were

performed using a fully randomized complete block design. In the control treatment, surface-sterilized tomato seeds (same surface-sterilization protocol as described previously) were soaked in sterile distilled water. One experimental treatment was performed by soaking surface-sterilized tomato seeds in 50 µg/ml of standard IAA solution as described by Shi et al. (2009) and the other consisted of soaking the surface-sterilized tomato seeds in the supernatant culture of *Streptomyces* sp. PT2 with IAA concentration adjusted to 50 µg/ml.

The effect of a presoaking period on the seed germination and root elongation was also studied for all treatments. The surface-sterilized seeds were soaked for 12, 24, 36, 48, 60 and 72 h and then cultivated in sterile sandy soil contained in plastic pots (12 cm height × 10 cm diameter).

The cultures were performed using six treated tomato seeds per pot and five replicates per treatment. Pots were then kept under greenhouse conditions (24 °C, 14 h light/10 h dark) and watered daily with 10 ml sterile tap water. Seed germination and root length were measured after 10 and 30 days respectively.

Statistical analysis

Data were subjected to a two-way ANOVA using SPSS software for Windows. Statistical differences between means were compared using Tukey's HSD (Honestly Significant Difference) test at $P = 0.05$.

Results

Effectiveness of the surface sterilization protocol

Twenty-seven actinomycete strains were isolated from the roots of the plants under study and purified. Representative samples from all actinomycete cultures treated with the surface sterilization protocol showed no microbial growth on chitin-vitamin B agar. In addition, the wash water samples from all surface-sterilized roots failed to grow on the same medium. This proves that epiphytic actinomycetes were eliminated and could not grow after the surface sterilization protocol. Consequently, the actinomycete strains obtained were indeed endophytic.

Preliminary identification of endophytic actinomycetes

All actinomycetes isolated were aerobic, spore forming, Gram positive and formed extensively branched hyphae. According to the morphological characteristics and diaminopimelic acid isomer analysis, twenty-two endophytic actinomycetes belonged to the *Streptomyces* genus (Table 1). Based on their substrate hyphae and spore mass colors on ISP3 medium, *Streptomyces* isolates were classified in three different groups. However, the spore-chain type S represented 77 % of endophytic *Streptomyces* strains and the majority of them showed grey spore masses. The five remaining strains had *meso*-diaminopimelic acid isomers and were classified under non-*Streptomyces* genera (Table 1).

Screening for Indole-3-acetic acid production

The twenty-seven endophytic isolates were screened for their IAA production abilities on YT broth supplemented with L-tryptophan. Eighteen strains were found to be producers of IAA and the production ranged from 12.28 to 100.03 µg/ml (Table 2). All roots except those of *P. turgidum* hosted endophytic actinomycetes strains unable to produce IAA. The supernatant culture of the strain producing the most IAA (*Streptomyces* sp. PT2) was used for IAA extraction and TLC characterization. Chromatograms of produced IAA spots and authentic IAA, revealed with Ehmann's reagent, showed almost the same R_f values. For this, *Streptomyces* sp. PT2 was selected for the molecular identification and optimization of culture conditions.

Molecular identification of *Streptomyces* sp. PT2

The molecular taxonomy and phylogenetic analysis using the 16S rDNA sequence for the strain producing the most IAA confirmed that the isolate PT2 belonged to the genus

Table 1 Morphological characteristics and diaminopimelic acid isomers of endophytic actinomycetes isolated from Saharan spontaneous plants

Groups	Color of spore mass	Substrate hyphae	Diffusible pigment	Spore-chain type	DAP isomers	Number of isolates (putative genus)
A	Colorless or pale yellow	Grey	Without	S	LL	15 (*Streptomyces*)
B	Colorless or pale yellow	Yellow	Without or yellow	RF	LL	5 (*Streptomyces*)
C	Orange	Green	Without	S	LL	2 (*Streptomyces*)
D	Colorless or pale yellow	White	Without	–	DL	4 (non-*Streptomyces*)
E	White	White	Without	–	DL	1 (non-*Streptomyces*)
Total						27

Table 2 IAA production by endophytic actinomycete strains after 5 days of incubation

Plant species	Endophytic actinomycete strain	Genus	IAA production (μg/ml)[a]
Cleome arabica	CA2	*Streptomyces*	17.92 ± 0.15
	CA3	*Streptomyces*	00.00
	CA6	*Streptomyces*	44.85 ± 0.65
	CA7	Non-*Streptomyces*	00.00
	CA9	*Streptomyces*	30.01 ± 0.23
	CA10	Non-*Streptomyces*	00.00
	CA11	Non-*Streptomyces*	12.28 ± 0.49
	CA12	*Streptomyces*	21.55 ± 0.42
	CA13	Non-*Streptomyces*	25.93 ± 0.36
Solanum nigrum	SN2	*Streptomyces*	27.03 ± 0.77
	SN3	*Streptomyces*	00.00
	SN5	*Streptomyces*	21.01 ± 1.71
	SN6	*Streptomyces*	65.42 ± 0.56
	SN7	*Streptomyces*	15.24 ± 0.30
	SN8	*Streptomyces*	22.15 ± 0.38
	SN10	Non-*Streptomyces*	00.00
	SN11	*Streptomyces*	44.07 ± 0.53
Aristida pungens	AP1	*Streptomyces*	16.04 ± 0.40
	AP2	*Streptomyces*	00.00
	AP3	*Streptomyces*	00.00
	AP5	*Streptomyces*	26.70 ± 0.47
	AP6	*Streptomyces*	00.00
	AP7	*Streptomyces*	37.36 ± 0.46
Panicum turgidum	PT1	*Streptomyces*	22.42 ± 0.38
	PT2	*Streptomyces*	100.03 ± 0.34
Astragallus armatus	AR1	*Streptomyces*	00.00
	AR2	*Streptomyces*	17.36 ± 1.01

[a] Average ± standard deviation from three replicates

Streptomyces. Its taxonomy based on 16S rDNA analysis within the *Streptomyces* genus is shown in Fig. 1. The similarity level was 99.52 % with *Streptomyces enissocaecilis* NRRL B 16365^T, *Streptomyces rochei* NBRC 12908^T and *Streptomyces plicatus* NBRC 13071^T, the most closely related species.

Optimization of *Streptomyces* sp. PT2 culture conditions

Culture experiments were carried out with different values of incubation period, temperature, pH, and L-tryptophan concentration for highest IAA production by *Streptomyces* sp. PT2. The effect of incubation periods, represented in Fig. 2, showed that IAA production started after 24 h, increased gradually and reached a maximum (3.63 μg/mg dry cell weight) after 7 days of incubation. Beyond this time, IAA production decreased gradually and reached 3.27 μg/mg dry cell weight after 10 days of incubation.

The effect of incubation temperature on the bacterial growth and IAA production was studied in the range of 20 to 40 °C (Fig. 3). *Streptomyces* sp. PT2 growth and IAA production were strongly influenced by incubation temperatures. Results showed that incubation at 25 °C gave the maximum IAA production (4.36 μg/mg dry cell weight).

The data in Fig. 4 show the effect of pH on the bacterial growth and IAA production. The production of IAA by *Streptomyces* sp. PT2 was maximum at pH 7.0. Acidity and high alkalinity of YT broth led to a marked decrease of growth and IAA production.

The results of the screening of *Streptomyces* sp. PT2 growth and IAA production in YT broth supplemented with different concentrations of L-tryptophan are represented in Fig. 5. The strain studied was able to produce IAA without supplementary L-tryptophan and the highest production (3.83 μg/mg dry cell weight) was obtained with 5 mg L-tryptophan/ml. IAA production decreased slightly with higher L-tryptophan concentrations.

Effect of IAA treatments and presoaking periods on seed germination and root elongation

The effects on seed germination and root elongation of treating tomato seeds with sterile distilled water, standard IAA and crude IAA in the supernatant culture of *Streptomyces* sp. PT2 for different presoaking periods are represented in Fig. 6. Seeds presoaked for different periods showed different seed germination rates (Fig. 6a) and root lengths (Fig. 6b). Treatments of 24 h in standard IAA and produced IAA had the highest effects on seed germination rate and root length of seedlings and no significant differences were found between these two treatments. However, reducing or extending the treatment periods caused significant diminutions ($P < 0.05$) of the measured parameters. Compared to controls, presoaking of tomato seeds in standard IAA and produced IAA for 24 h showed significant differences ($P < 0.05$) for both the seed germination rates and root lengths (Fig. 6b).

Discussion

In the present study, 27 actinomycete strains were isolated from the roots of five selected spontaneous plants of the Algerian Sahara. To eliminate epiphytic isolates, all tested strains were proved to be associated with plants and were isolated from inside the roots of the plants studied. Coa et al. (2005), Shi et al. (2009), Ruanpanun et al. (2010) have reported the colonization of many plant roots by beneficial actinomycetes.

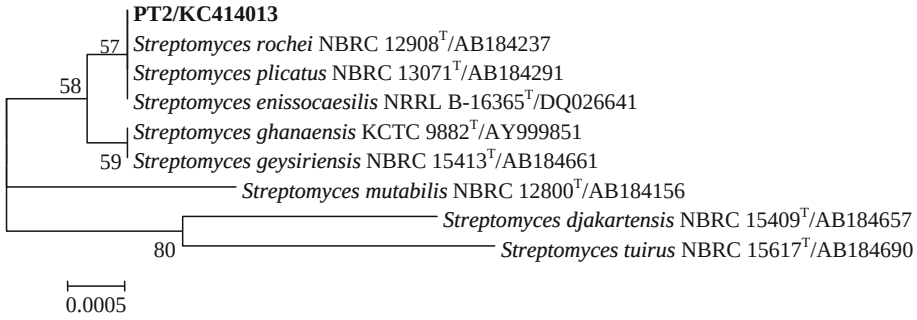

Fig. 1 Phylogenic tree based on 16S rDNA gene sequences showing relationships among *Streptomyces* sp. strain PT2 and the most-closely related type-strain species of *Streptomyces*. *Numbers at nodes* indicate percentages of bootstrap support based on a neighbor-joining analysis of 1,000 resampled datasets; only values above 50 % are given. *Bar* 0.0005 substitutions per nucleotide position

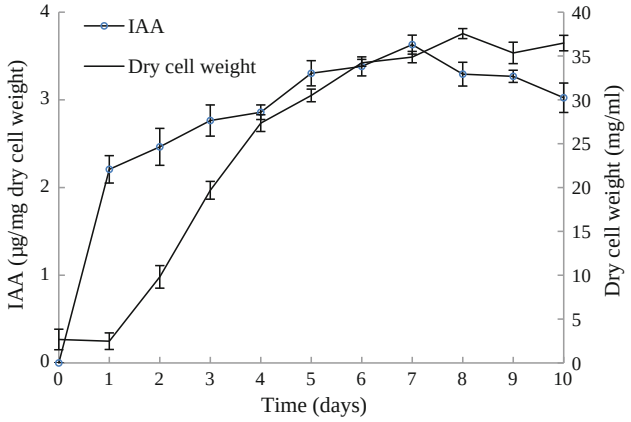

Fig. 2 Production of indole-3-acetic acid (IAA) by *Streptomyces* sp. PT2 on yeast extract-tryptone broth for different incubation periods

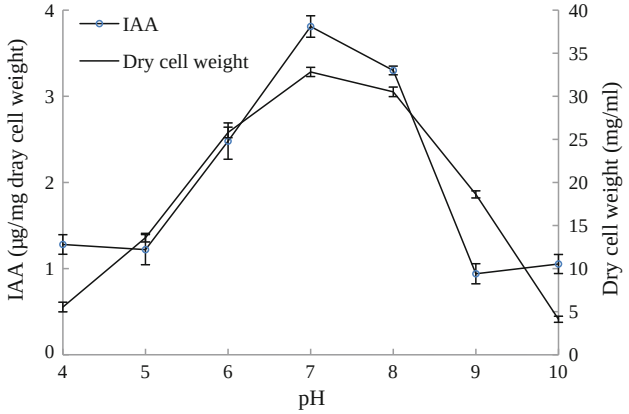

Fig. 4 Production of indole-3-acetic acid (IAA) by *Streptomyces* sp. PT2 on yeast extract-tryptone broth at different pH

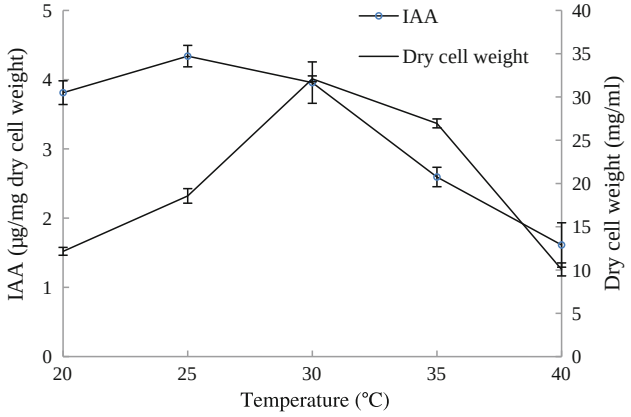

Fig. 3 Production of indole-3-acetic acid (IAA) by *Streptomyces* sp. PT2 on yeast extract-tryptone broth at different temperatures

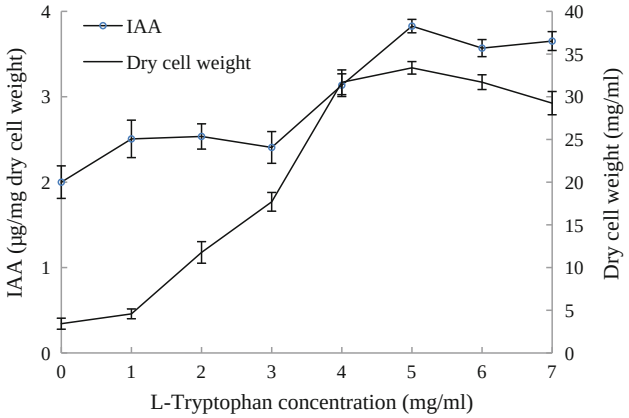

Fig. 5 Production of indole-3-acetic acid by *Streptomyces* sp. PT2 on yeast extract-tryptone broth with different concentrations of L-tryptophan

The majority of endophytic actinomycete strains were classified in the *Streptomyces* genus, which is the most representative of actinomycetes in the soil (Goodfellow and Simpson 1987). *Streptomyces* is also the most dominant endophytic genus in the roots of plants such as tomato (Coa et al. 2004), banana (Coa et al. 2005) and some medicinal plants (Qin et al. 2009).

Non-*Streptomyces* species were also found to colonize the roots of the plants studied. Qin et al. (2009) reported that non-*Streptomyces* species (*Amycolatopsis* and *Nocardia* genera) were isolated from the surface-sterilized roots of many plants.

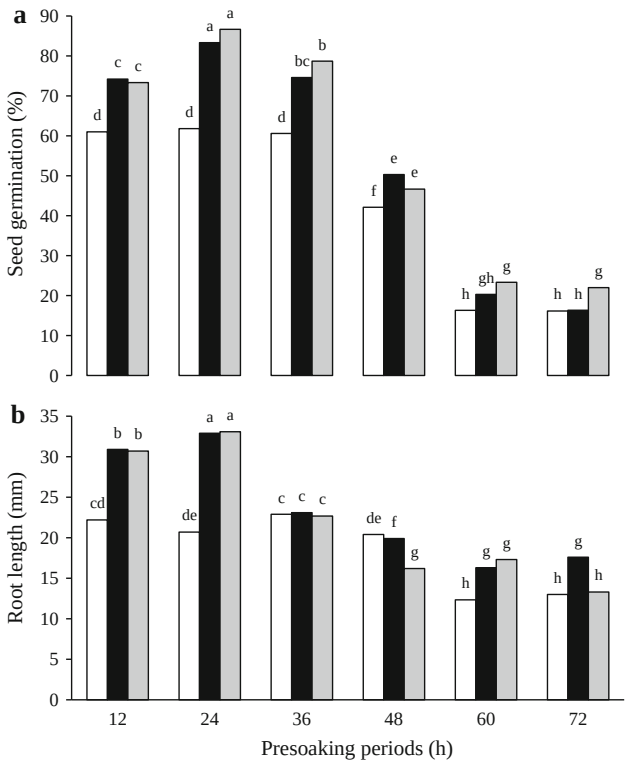

Fig. 6 Effect presoaking periods of tomato seeds in sterile distilled water (*white bars*), standard IAA (*black bars*) and produced IAA (*grey bars*) on the seed germination (**a**) and root length (**b**). *Bars labeled with different letters* indicate significant difference between treatments according to Tukay's HSD test at $P = 0.05$

The screening for IAA production (Table 2) showed that the majority of strains were able to produce this plant growth regulator and all root samples hosted at least one IAA-producing strain. However, several papers have reported that endophytic actinomycetes isolated from various plants are able to produce plant growth regulators, especially IAA (Aldesuquy et al. 1998; Tokala et al. 2002; El-Tarabily et al. 2008).

The strain producing the most IAA (*Streptomyces* sp. PT2) was isolated from the roots of *P. turgidum*. Tokala et al. (2002) and El-Tarabily et al. (2008) reported that roots could contain high concentrations of L-tryptophan in their tissues and support the activity of endophytic *Streptomyces* to produce IAA. Endophytic *Streptomyces* producing IAA can strongly promote plant growth by increasing root elongation and through several other beneficial effects (Aldesuquy et al. 1998; Tokala et al. 2002).

The literature reports no research on endophytic *Streptomyces* isolated from the roots of *P. turgidum* and this is the first description of IAA production by this endophytic species, which was selected for the molecular taxonomy.

The maximum IAA production (127 µg/ml) by *Streptomyces* sp. PT2 is comparable with results by Khamna et al. (2010), who reported that *Streptomyces viridis* CMU-H009 produced 143.95 µg/ml. However, IAA production by *Streptomyces* sp. PT2 is significantly higher than by *Streptomyces* sp. CMU-MH021, which produces only 28.5 µg/ml (Ruanpanun et al. 2010).

The effect of incubation periods, temperature, pH and L-tryptophan concentrations were investigated in order to optimize the culture conditions for highest IAA production by *Streptomyces* sp. PT2. The maximum of production was reached after 7 days of incubation (Fig. 2). Decreasing IAA production after this incubation period might be explained by the release of degraded IAA due to oxidase and peroxidase activities as has been shown in *Rhizobium* sp. from *Cajanus cajan* (Datta and Basu 2000).

Incubation of cultures at 25 °C promoted IAA production (Fig. 3). These findings are in agreement with those of Aldesuquy et al. (1998), who reported that temperatures in the range of 25–30 °C were suitable for growth and IAA production by *Streptomyces* spp.

Neutral pH favoured IAA production (Fig. 4). Acidic or high alkaline pH decreased IAA production because *Streptomyces* species grow poorly in these pH conditions. In the natural environment, it is known that the distribution of *Streptomyces* spp. in acidic and highly alkaline soils is lower than in neutral soils (Shirokikh et al. 2007). The acidic and very alkaline pH affects the function of enzyme systems and the solubility of many substances that are necessary for bacterial growth and IAA production. The optimum pH found here for highest IAA production by *Streptomyces* sp. PT2 is in agreement with other reports (Yurekli et al. 2003; Ahmad et al. 2005; Khamna et al. 2010).

IAA production was strongly influenced by L-tryptophan concentration. *Streptomyces* sp. PT2 produced IAA on L-tryptophan-free YT broth. This production was also found for *Azotobacter* and *Pseudomonas* isolates cultured without L-tryptophan sources (Ahmad et al. 2005). However, Khamna et al. (2010) reported that *Streptomyces viridis* CMU-H009 was unable to produce IAA without addition of L-tryptophan. In the case of *Streptomyces* sp. PT2, a significant increase in growth and IAA production occurred with increasing L-tryptophan concentration. Ahmad et al. (2005) reported some evolution of kinetics in presence of high amounts of L-tryptophan, whereas high concentrations of tryptophan had a reverse effect on IAA production. Furthermore, Khamna et al. (2010) deduced a significant decrease in IAA production by *Streptomyces viridis* CMU-H009 when a high level of tryptophan was used. This effect of catabolite repression on production pathways is well known in bacteria, and mostly present in metabolite biosynthesis pathways.

The effect of seed treatments with IAA on the promotion of seed germination and root elongation of tomato is highlighted in Fig. 6. El-Tarabily (2008) reported that

Streptomyces spp. from tomato rhizosphere had the ability to produce IAA and improve tomato growth by increasing root dry weight. Furthermore, other reports have confirmed that endophytic actinomycetes produce IAA and promote seed germination and root elongation of many plants such as *Triticum aestivum* (Aldesuquy et al. 1998), *Sebsania aculeata* (Ahmad et al. 2005), *Zea mays* and *Bruguiera parviflora*a (Khamna et al. 2010), and *Ochetophila trinervis* (Solans et al. 2011).

Our results are similar to those of El-Tarabily et al. (2008), which mention that the effects of auxins on plant seedlings are concentration dependent. Low concentration may stimulate growth while high concentration may be inhibitory.

Presoaking of tomato seeds for 24 h in standard IAA and produced IAA solutions (50 µg/ml) were the most important seed treatments. These findings are in agreement with results reported by Shi et al. (2009), who showed a significant promotion of plant growth in sugar beet treated with a small quantity of IAA. Presoaking of tomato seeds for 36–72 h showed a considerable decrease in seed germination rate and root elongation of seedlings. These observations are supported by many reports concerning these adverse effect on the plant growth in wheat (Aldesuquy et al. 1998), pea (Kukavica et al. 2007), maize and cowpea (Ahmad et al. 2005) using high-IAA seed treatments.

The present study indicates that the five Saharan spontaneous plants from Algeria could provide interesting sources of endophytic actinomycetes producing IAA. The suitability of the *Streptomyces* sp. PT2 strain for IAA production and growth promotion activities will be studied with other important crops. Other growth promotion attributes that are exhibited by this strain, such as Gibberellic acid production, siderophore production, nutrient solubilization and uptake enhancement, also need to be investigated.

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