Biocontrol of *Rhizoctonia solani* damping-off and promotion of tomato plant growth by endophytic actinomycetes isolated from native plants of Algerian Sahara☆

Yacine Goudjal[a,b], Omrane Toumatia[a], Amine Yekkour[a], Nasserdine Sabaou[a], Florence Mathieu[c], Abdelghani Zitouni[a,*]

[a] *Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, Alger, Algeria*
[b] *Département d'agronomie, Faculté des Sciences, Université Amar Telidji, BP 37G, Laghouat 03000, Algeria*
[c] *LGC UMR 5503 (CNRS/INPT/UPS), Département de Bioprocédés et Systèmes Microbiens, ENSAT-INP de Toulouse, Université de Toulouse, 1 Avenue de l'Agrobiopôle, BP 32607, 31326 Castanet-Tolosan Cedex 1, France*

ARTICLE INFO

Keywords:
Endophytic actinomycetes
Biocontrol
Rhizoctonia solani
Damping-off
Tomato

ABSTRACT

Thirty-four endophytic actinomycetes were isolated from the roots of native plants of the Algerian Sahara. Morphological and chemical studies showed that twenty-nine isolates belonged to the *Streptomyces* genus and five were non-*Streptomyces*. All isolates were screened for their *in vitro* antifungal activity against *Rhizoctonia solani*. The six that had the greatest pathogen inhibitory capacities were subsequently tested for their *in vivo* biocontrol potential on *R. solani* damping-off in sterilized and non-sterilized soils, and for their plant-growth promoting activities on tomato seedlings. In both soils, coating tomato seeds with antagonistic isolates significantly reduced ($P < 0.05$) the severity of damping-off of tomato seedlings. Among the isolates tested, the strains CA-2 and AA-2 exhibited the same disease incidence reduction as thioperoxydicarbonic diamide, tetramethylthiram (TMTD) and no significant differences ($P < 0.05$) were observed. Furthermore, they resulted in a significant increase in the seedling fresh weight, the seedling length and the root length of the seed-treated seedlings compared to the control. The taxonomic position based on 16S rDNA sequence analysis and phylogenetic studies indicated that the strains CA-2 and AA-2 were related to *Streptomyces mutabilis* NBRC 12800^T (100% of similarity) and *Streptomyces cyaneofuscatus* JCM 4364^T (100% of similarity), respectively.

1. Introduction

Rhizoctonia solani is one of the most important soil-borne fungal pathogens, which develops in both cultured and non-cultured soils (Coa et al. 2004). It lives in the soil in the form of sclerotia and does not generate asexual spores (Huang et al. 2011). Damping-off of seedlings is the most common disease caused by *R. solani* (Moussa 2002). It has a wide host range and causes disease in a variety of crops, such as lawn grass (Parmeter et al. 1969), tomato (Coa et al. 2004), cucumber (Coa et al. 2004) and sugar beet (Sadeghi et al. 2006).

Chemical fungicides are often used when losses from *R. solani* are substantial. They have fungicidal activity against the pathogen or they are converted into toxic derivatives by the pathogen or host plant tissue (Grissbuhler et al. 1982). The intensive use of chemical fungicides has not only created problems of fungicide resistance and increased contamination of the soil, but may also have adverse high toxicity on microbial communities and a degradative effect on the ozone layer. In addition, chemical controls are not completely effective, and *Rhizoctonia* disease remains a persistent problem (Gees and Coffe 1989; Huang et al. 2011).

The success of *Bacillus subtilis* as a potential biocontrol agent encouraged research into new microbial agents as alternatives to chemical control compounds. In recent years, the biocontrol of plant diseases, particularly using antibiotic metabolites of filamentous bacteria, has been put forward as an alternative to chemical control agents (Dhanasekaran et al. 2005; Huang et al. 2011). The role of actinomycetes in the biocontrol of soil-borne plant pathogens has been demonstrated against various pathogens such as *Fusarium* spp. (Sabaou and Bounaga 1987; Gopalakrishnan et al. 2011), *Phytophthora* spp. (Shahidi Bonjar et al. 2006), *Pythium* spp. (Hamdali et al. 2008), *Rhizoctonia* spp. (Sadeghi et al. 2006), and *Verticillium* spp. (Meschke and Schrempf 2010).

Actinomycetes can occur in the plant rhizosphere soil and exercise an antagonistic and competitive effect on the microbial communities. They have the ability to produce active compounds, such as antifungal and antibacterial antibiotics or plant growth

☆ This article is part of a Special Issue entitled Plant Growth Promotion.
* Corresponding author. Tel.: +213 21 29 75 11; fax: +213 21 28 20 67.
E-mail address: zitouni_abdelghani@yahoo.fr (A. Zitouni).

http://dx.doi.org/10.1016/j.micres.2013.06.014

regulators (PGRs), that have been developed for agricultural uses (Suzuki et al. 2000; Ilic et al. 2007). They have also been used as commercially formulated biocontrol agents of plant diseases such as *Streptomyces griseoviridis* cells used to protect crops against infections by *Fusarium* spp. and *Alternaria* spp. (Lahdenperä et al. 1991). In addition to their ability to inhibit plant pathogens, some actinomycetes are also known to form close associations with plants, colonize their internal tissues without causing disease symptoms, and promote their growth (Kunoh 2002).

Several native plants have successfully adapted to the stressful conditions of the Algerian Sahara, especially the poor sandy soil and the drought of the arid climate. They grow and colonize these areas annually without human intervention (Pouget 1980). The success of natural regeneration from seeds and the vigorous growth of native plants under the harsh conditions of the Sahara could suggest a contribution of endophytic microbes to the biological protection of germinated seeds against soil-borne pathogens and to the promotion of plant growth. In recent work, we initiated a study to highlight the role of endophytic microbes from these plant species as potential biocontrol agents against damping-off disease caused by soil-borne pathogens and as plant growth promoters (Goudjal et al. 2013).

The aims of the present study were to isolate endophytic actinomycetes from healthy Saharan plants and bring out their potential role on the biocontrol of *R. solani* damping-off and on plant growth promoting activities for tomato seedlings. In order to select the most interesting strains for the biocontrol of *R. solani* damping-off, a commercial chemical agent was used as a control.

2. Materials and methods

2.1. Sample collection and endophytic actinomycetes isolation

Healthy native plants (Aristida pungens, Cleome arabica, Solanum nigrum, Panicum turgidum, *Astragallus armatus*, *Peganum harmala*, *Hammada scoparia* and *Euphorbia helioscopia*) were collected from the Laghouat region in the northern Algerian Sahara (33°44′ N, 2°47′ E) in March 2011. The selection of plant species for endophyte isolation was based on their abundance and adaptation to the poor sandy soil and arid climatic conditions of the Algerian Sahara. Five healthy root samples were harvested from each plant species. No record indicates that these plants have ever been studied for endophytic actinomycetes isolation previously.

Endophytic actinomycetes were isolated according to the method of Taechowisian et al. (2003). The culture medium used was chitin-vitamin agar (Hayakawa and Nonomura 1987) supplemented with cycloheximide (80 mg l^{-1}) and nalidixic acid (15 mg l^{-1}) to suppress the growth of fungi and Gram-negative bacteria, respectively.

2.2. Effectiveness of the surface-sterilization protocol

To prove that epiphytic actinomycetes could not grow after the surface-sterilization of the roots and that all the isolated actinomycetes were endophytic, the method described by Schulz et al. (1993) was used.

2.3. Identification of the endophytic actinomycetes

Endophytic actinomycetes belonging to the *Streptomyces* genus were identified based on cultural and micromorphological characteristics according to the methods of Shirling and Gottlieb (1966) and Goodfellow and Simpson (1987).

In order to distinguish the *Streptomyces* genus, the cell wall type was determined by a chemical study based on the occurrence of diaminopimelic acid isomers (Becker et al. 1964).

Identification of the highest potential isolates (CA-2 and AA-2) on biocontrol of *R. solani* damping-off and plant growth promoting activity for tomato *cv*. Marmande seedlings was confirmed by the 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 as used by Boubetra et al. (2013). The PCR products obtained were sent to the MilleGen Company (Toulouse, France) for sequence determination. The16S rRNA sequences have been deposited in the GenBank data library and assigned the accession numbers KC414006 and KC414004 for CA2 and AA2 strains, respectively. The obtained sequences were compared with sequences present in the public sequence databases and with EzTaxon tools (Chun et al. 2000).

2.4. In vitro antagonism assay

The streak method described by Boubetra et al. (2013) was used to test the antifungal activity of the endophytic actinomycete isolates against an indigenous soil-borne pathogenic strain of *R. solani* LRS1 isolated from tomato fields in the Laghouat region (33°46′ N, 2°50′ E). Inhibition zones were evaluated as follows: (<5 mm) no inhibition, (5-9 mm) weak inhibition, (10-19 mm) moderate inhibition, and (≥20 mm) strong inhibition.

2.5. In vivo biocontrol trials

2.5.1. Soil properties

The sandy soil used to test the effectiveness of the antagonistic isolates to control damping-off of tomato *cv*. Marmande was collected from a tomato field in the Laghouat region (33°46′ N, 2°50′ E). Its physicochemical and biological characteristics were: pH 7.8, total organic matter (1.8%), C/N (9.3), phosphate (0.07‰), potash (0.3‰), $CaCO_3$ (1.1%) total aerobic bacterial count 1.52×10^8 CFU g^{-1} and total fungal count 3.7×10^4 CFU g^{-1}. Soil was sterilized three times in an autoclave at 120 °C for 60 min on three consecutive days.

2.5.2. Preparation of microbial suspensions

Actinomycete suspensions were prepared as described by Errakhi et al. (2007). Tween-80 solution (0.05%) was used to recover the spores, which were adjusted to 10^6 CFU ml^{-1} by the same solution using the Thomas cell. A pathogen suspension was prepared by growing *R. solani* in PDA dishes at 25 °C for 10 days. Spores were directly recovered in sterile distilled water and adjusted to 10^5 CFU ml^{-1} in the same way.

2.5.3. Coating of tomato seeds

Tomato seeds were surface-sterilized using the method of Coa et al. (2004). Sterilized seeds were coated separately by soaking for 30 min in the actinomycete spore suspensions and then dried under a laminar flow hood. Actinomycete spores on the coated seeds yielded approximately 10^7 CFU g^{-1}.

2.5.4. Preparation of infested soils

Sterilized and non-sterilized soils were infested according to a method similar to that used by Dhanasekaran et al. (2005). Soils were conditioned in plastic pots (12 cm high × 10 cm in diameter) and infested with 10 ml of pathogen suspension diluted in 90 ml sterile distilled water (100 ml sterile distilled water for uninfected soils). The pots were covered with plastic film and incubated for one week at room temperature to promote pathogen growth. *R. Solani* density in the infested soils was evaluated at 10^4 CFU g^{-1}.

2.5.5. Biocontrol assays

Based on their *in vitro* antifungal activities, six antagonistic isolates were selected for *in vivo* biocontrol of damping-off of tomato *cv.* Marmande seedlings caused by *R. Solani*. Experiments were carried out in both sterilized and non-sterilized soils, where four treatments were performed: (1) positive control by sowing uncoated tomato seeds in non-infested soils; (2) negative control by sowing uncoated tomato seeds in infested soils to evaluate the varietal sensitivity; (3) sowing coated tomato seeds in infested soils to evaluate the biocontrol capacity of each antagonistic isolate against *R. solani* damping-off; (4) use of a chemical agent, thioperoxydicarbonic diamide, tetramethylthiram (TMTD), to control *R. solani* damping-off. In the last case, sterilized seeds were treated as recommended by the manufacturer (thorough mixing, for 3 min, of 10 g of the surface-sterilized tomato seeds in 0.08 g of chemical control agent wetted with 10 ml of distilled water and drying for 2 h under a laminar flow hood), before being cultivated in infested soils.

For each treatment, six tomato seeds were sown per pot at 5 mm from the soil surface, with ten replicates per treatment using a fully randomized complete block. The tomato cultures were grown under standard conditions in a plant growth room (22-26 °C, 16 h light/8 h dark). Pots were watered daily with 10 ml tap water to maintain a moisture level favorable for seed germination.

After 30 days, seedlings were carefully removed from the soil and washed with tap water. The disease severity was rated using a 5-class scale as described by Altier and Thies (1995).

2.6. Internal root colonization abilities

The method described by Misaghi and Donndelinger (1990) was used to study the internal root colonization abilities of endophytic actinomycete strains that significantly decreased the incidence of damping-off and significantly promoted the growth of tomato seedlings.

2.7. Statistical analysis

A one-way analysis of variance (ANOVA) was performed on the data and significant differences between means were compared using Fisher's protected LSD test at $P = 0.05$. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Validity of the surface-sterilization protocol

All cultures of endophytic actinomycetes treated with the surface-sterilization protocol were unable to grow on chitin–vitamin agar medium. This indicates that the isolates tested were endophytic and the epiphytic ones could not grow after the sterilization protocol.

3.2. Identification of endophytic actinomycetes

Thirty-four endophytic actinomycetes were isolated from the eight native Algerian Saharan plants. The morphological characteristics and the cell wall type (Table 1) confirmed that the majority of isolates (29 isolates) belonged to the genus *Streptomyces*. Endophytic *Streptomyces* strains were isolated from all studied native plants. They were classified in three groups according to the color of the aerial hyphae on ISP3 agar. The majority of them (22 isolates) belonged to the grey group. The yellow and the green groups comprised 5 and 2 isolates, respectively. The majority of the *Streptomyces* isolates (82.8%) produced a spiral type of spore-chain and the remaining ones (17.2%) produced a *rectiflexibiles* type.

Table 1
Morphological and chemical characteristics of isolated endophytic actinomycetes.

Host plants	Color of			Spore-chain types	DAP isomers	Genus	Number of isolates
	Substrate hyphae	Aerial hyphae	Diffusible pigments				
Aristida pungens	Colorless	Grey or brownish grey	Without	S	LL	*Streptomyces*	5
	Strong reddish brown	Greenish grey	Without	S	LL	*Streptomyces*	2
Cleome arabica	Moderate olive	Light brownish grey	Without	S	LL	*Streptomyces*	5
	Colorless	White or Yellowish white	Without	/	DL	non-*Streptomyces*	4
Solanum nigrum	Colorless	Medium grey	Without	S	LL	*Streptomyces*	5
	Light yellowish brown	Yellowish grey	Brownish orange	RF	LL	*Streptomyces*	3
Panicum turgidum	Colorless	Light brownish grey	Without	S	LL	*Streptomyces*	2
Astragallus armatus	Moderate olive	Grayish yellow	Without	RF	LL	*Streptomyces*	2
Peganum harmala	Colorless	Light brownish grey	Without	S	LL	*Streptomyces*	1
	Colorless	Yellowish white	Without	/	DL	non-*Streptomyces*	1
Hammada scoparia	Light brownish grey	Brownish grey	Without	S	LL	*Streptomyces*	2
Euphorbia helioscopia	Colorless	Medium grey	Without	S	LL	*Streptomyces*	2
Total							34

S, *Spira*; RF, *Rectus flexibilis*; DAP, diaminopimelic acid.

Non-*Streptomyces* isolates made up 14.7% of all the endophytic isolates and were obtained only from the roots of *Cleome arabica* and *Hammada scoparia*.

The isolates CA-2 and AA-2, which showed the highest biocontrol capacities on *R. solani* damping-off and the highest growth promoting activities on tomato seedlings, were selected for the molecular taxonomy analysis (GenBank KC414006 and KC414004, respectively). Taxonomically, the strains CA-2 and AA-2 were ranked in two different morphological groups and they were related to *Streptomyces mutabilis* NBRC 12800^T (100% similarity) and *Streptomyces cyaneofuscatus* JCM 4364^T (100% similarity), respectively on the basis of the 16S rRNA gene comparison.

According to the aerial spore mass color, the strain CA-2 belonged to the Grey series of the genus *Streptomyces*. The spore chains produced were in the section *Spirales*, and contained 10-50 spores per chain. This strain was compared to the phylogenetically related type strain NBRC 12800^T of *S. mutabilis*. As described by Kämpfer (2012), this species can be ranked in both the Grey and White series and can produces spore chains in the *Spirales* or *Retinaculiaperti* sections. Furthermore, spore chains are poorly developed, with shorter hooks, loops, or partial spirals, and contain only 3-10 spores per chain.

The strain AA-2 belonged to the Yellow series of the genus *Streptomyces*, and the spore chains produced were in the section *Rectiflexibilis*. Mature spore chains contained 10-50 spores per chain. The strain has the same morphological characteristics as its phylogenetically related type strain JCM 4364^T of *S. cyaneofuscatus* (Kämpfer, 2012).

The both species of *Streptomyces*, *S. mutabilis* and *S. cyaneofuscatus* have never been reported for their biocontrol and growth promoting activities.

3.3. *In vitro antagonistic activities*

Twenty-one isolates of the genus *Streptomyces* showed an antifungal activity against *R. solani* (Table 2) but only six of them strongly inhibited (inhibition zone >20 mm) the mycelium growth of the pathogen. The remaining isolates failed to induce, or induced only small, inhibition zones (<20 mm) and so were not included in subsequent studies. The antagonistic effects appeared after two days of paired incubation of actinomycetes and fungi. This attribute suggests that some of the isolates tested may be efficient in controlling damping-off caused by *R. solani*. Based on the mean size of the inhibition zones, the *Streptomyces* strains AP-4, AP-5, CA-2, SN-5, SN-10 and AA-2 were selected for *in vivo* biocontrol of *R. solani* damping-off of tomato seedlings.

3.4. *In vivo biocontrol of R. solani damping-off of tomato seedlings*

High disease incidence (90.3% and 88.5%) was observed with untreated tomato seeds in sterilized and non-sterilized soils, respectively (Fig. 1A). After the seeds had germinated, the rootlets were subjected to the effects of *R. solani*. Considerable root rot was observed in the newly germinated seeds and caused their death (Fig. 2A). The root rot symptoms were also observed in seedling roots. They evolved and led to desiccation of the roots and damping-off of the seedlings (Fig. 2b and c). However, normal growth and healthy seedling roots were obtained for the tomato seeds coated with spores of the strain *Streptomyces* sp. CA-2 growing in the soil infested by *R. solani* (Fig. 2d).

For the majority of seed treatments, the protective effects were more pronounced in sterilized soil than in non-sterilized soil (Fig. 1A). The compound TMTD and the biocontrol agents (CA-2, AA-2, AP-4, SN-10, SN-5 and AP-5) allowed a significant reduction in disease incidence ($P < 0.05$) in seed-treated seedlings. Healthy

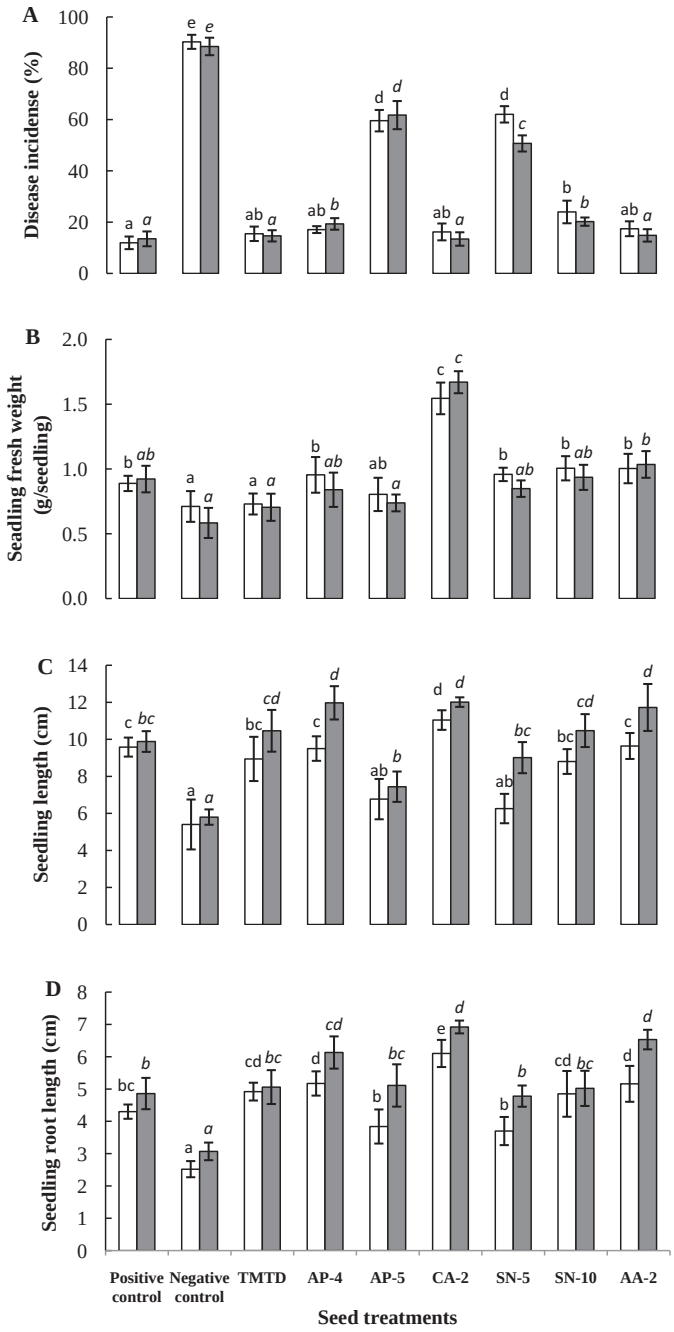

Fig. 1. Effect of seed treatment with TMTD and spore suspensions of antagonistic actinomycetes (AP-4, AP-5, CA-2, SN-5, SN-10 and AA-2) on the disease incidence (A), seedling fresh weight (B), seedling length (C) and root length (D) in sterilized (*white bars*) and non-sterilized (*grey bars*) soils. Evaluation was made 30 days after planting. Bars labeled with the same letters are not significantly different according to Fisher's protected LSD test at $P = 0.05$.

seedling rates obtained by these treatments varied from 38.0% to 86.6% compared to the negative control that exhibited only 19.7% of healthy seedlings. The strains CA-2 and AA-2 and the compound TMTD had the greatest incidence of disease reduction in both sterilized and non-sterilized soils (86.6%, 85.3% and 85.2% healthy seedlings, respectively) and no significant differences ($P < 0.05$) were observed among them. However, the biocontrol capacities of the remaining strains AP-4, AP-5, SN-5 and SN-10 were less than that of TMTD. The rates of the healthy tomato seedlings obtained by these treatments were 82.9%, 40.4%, 49.3% and 79.8%, respectively.

Table 2
In vitro antagonistic activity of endophytic actinomycetes against *Rhizoctonia solani* LRS1.

Host plants	Endophytic isolates	Genus	Diameter of inhibition zone (mm)[a]
Aristida pungens	AP-1	*Streptomyces*	0
	AP-2	*Streptomyces*	0
	AP-3	*Streptomyces*	0
	AP-4	*Streptomyces*	30 ± 1.5
	AP-5	*Streptomyces*	28 ± 0.6
	AP-6	*Streptomyces*	0
	AP-7	*Streptomyces*	0
Cleome arabica	CA-2	*Streptomyces*	35 ± 1.2
	CA-3	*Streptomyces*	0
	CA-6	*Streptomyces*	17 ± 0.6
	CA-7	non-*Streptomyces*	0
	CA-9	*Streptomyces*	19 ± 1.5
	CA-10	non-*Streptomyces*	16 ± 1.0
	CA-11	non-*Streptomyces*	15 ± 0.6
	CA-12	*Streptomyces*	17 ± 1.2
	CA-13	non-*Streptomyces*	0
Solanum nigrum	SN-2	*Streptomyces*	16 ± 0.6
	SN-3	*Streptomyces*	0
	SN-4	*Streptomyces*	14 ± 0.6
	SN-5	*Streptomyces*	28 ± 1.5
	SN-7	*Streptomyces*	14 ± 0.6
	SN-8	*Streptomyces*	17 ± 1.3
	SN-10	*Streptomyces*	36 ± 0.8
	SN-11	*Streptomyces*	16 ± 1.2
Panicum turgidum	PT-1	*Streptomyces*	19 ± 0.9
	PT-3	*Streptomyces*	18 ± 1.2
Astragallus armatus	AA-2	*Streptomyces*	30 ± 1.3
	AA-3	*Streptomyces*	14 ± 0.9
Peganum harmala	PH-1	*Streptomyces*	0
	PH-8	non-*Streptomyces*	0
Hammada scoparia	HS-1	*Streptomyces*	15 ± 1.5
	HS-2	*Streptomyces*	19 ± 0.8
Euphorbia helioscopia	EH-3	*Streptomyces*	0
	EH-4	*Streptomyces*	0

[a] Average ± standard deviation from three replicates.

In both soils, seed treatment with the strain *Streptomyces* sp. CA-2 exhibited the greatest growth promoting activities on tomato seedlings. Compared to the positive control, it significantly increased ($P < 0.05$) the seedling fresh weight from 0.92 g/seedling to 1.67 g/seedling (Figs. 1B and 3), the seedling length from 9.88 cm to 12.01 cm (Fig. 1C) and the root length from 4.86 cm to 6.92 cm (Fig. 1D). Nevertheless, smaller growth promoting activities were observed on tomato seedlings that received the treatments with spores of the *Streptomyces* spp. AP-4, AP-5, SN-5 and SN-10. Compared to the positive control, no important changes were exhibited in the promoting of seedling fresh weight (0.89 g/seedling to

Fig. 2. Tomato (*cv.* Marmande) seedlings grown in the *R. solani* infested soil without seed treatment (a)–(c) and by treatment of seeds with spores of the strain *Streptomyces* sp. CA-2 (d). Arrows indicate the root rot symptoms caused by *R. solani* on primary rootlet (a) and on seedling roots (b) and (c) compared to a healthy seedling root (d). Pictures were taken after 10 days in plant growth room under standard growth conditions.

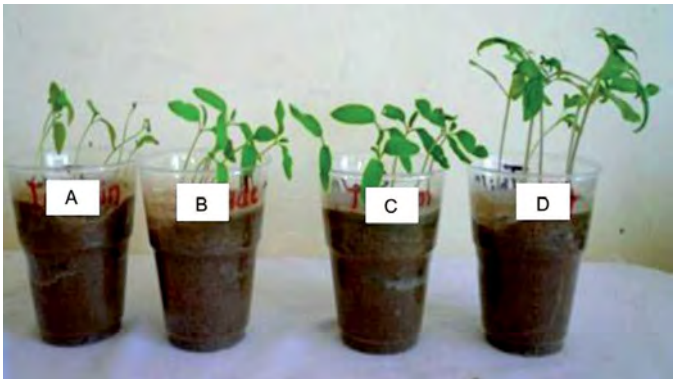

Fig. 3. Tomato *cv.* Marmande seedlings obtained by sowing uncoated tomato seeds in sterilized soil infested with *R. solani* as negative control (A), uncoated seeds sowed in sterilized soil as positive control (B), treated seeds with the chemical TMTD sowed in infested soil (C), and coated seeds with spores of the strain CA-2 in infested soil (D). The picture was taken after 14 days under standard growth conditions in plant growth room.

1.04 g/seedling), seedling length (9.58 cm to 10.47 cm) and seedling root length (4.30–5.02 cm).

3.5. Internal root colonization abilities

Results for the root colonization abilities of the six strains tested (AP-4, AP-5, CA-2, SN-5, SN-10 and AA-2) showed no visible growth of endophytic actinomycetes from the control seedlings. However, all strains were successfully isolated from the surface-sterilized roots of inoculated tomato seedlings. This indicates that these strains were endophytic and their endophytic colonizing abilities were demonstrated in both their indigenous host plants and inoculated tomato roots.

4. Discussion

Several studies have reported the use of actinomycete strains for biocontrol of *R. solani* damping-off (Moussa 2002; Coa et al. 2004; Chung et al. 2005; Sadeghi et al. 2006; Patil et al. 2010). In addition, commercial products to control crop damping-off, such as Mycostop® (*Streptomyces griseoviridis* strain K61) and Actinovate® (*Streptomyces lydicus* strain WYEC108), have been registered (Lahdenperä et al. 1991).

In this study, six endophytic actinomycete strains (AP-4, AP-5, CA-2, SN-5, SN-10 and AA-2) isolated from roots of native plants from the Algerian Sahara showed *in vitro* production of antifungal compounds with a large inhibition zone (≥28 mm) for *R. solani* mycelium growth. Their abilities to reduce the incidence of damping-off and to promote the growth of tomato seedlings in both sterilized and non-sterilized soils were noted.

The biocontrol of *R. solani* damping-off in both soils showed that all tested strains significantly ($P < 0.05$) reduced the disease incidence compared to the negative control (Figs. 1 and 3). The strains CA-2, AA-2, AP-4 and SN-10 reduced the disease incidence by over 75%. However, the disease reduction reached by the remaining tested strains (AP-5 and SN-5) was less than 33%.

Endophytic actinomycete strains selected on the basis of *in vitro* antagonistic activities were effective in controlling *R. solani* damping-off in both soils. Their biocontrol activities allowed 40.4% to 86.6% healthy seedlings compared to the negative control that exhibited only 9.7%. The plant protection could be resulted by the action of antagonistic actinomycete strains on the growth of *R. solani*. Several mechanisms can be involved, especially the production of antifungal compounds, the chitinolytic activities and the nutrient competition by the production of siderophores. However, many factors are likely to affect biocontrol of damping-off *in vivo*, including environmental conditions such as soil temperature, soil morphology, pH value, water status, nutrient availability and interactions with indigenous soil microbes (Dhanasekaran et al. 2005).

In the *in vivo* conditions, the rhizospheric microorganisms could also interact with inoculated antagonists, which influenced their biocontrol capacities. Our study showed that all antagonistic strains of *Streptomyces* tested reduced the disease incidence more in non-sterilized soil than in sterilized soil. The positive effect of interaction with indigenous soil microorganisms on the control of *R. solani* damping-off was also confirmed by Errakhi et al. (2007) in *Sclerotium rolfsii* damping-off of sugar beet with *Streptomyces* spp. Furthermore, as reported by Shen (1997) and Kennedy (1999), these findings suggest that the rhizospheric indigenous flora contributes to the control of *R. solani* disease and could have a synergic effect with the antagonistic actinomycetes.

This would suggest that several mechanisms are involved in controlling plant pathogens by endophytic actinomycetes in soil conditions. Trejo-Estrada et al. (1998) reported that antibiosis is likely to be the important mechanism as the majority of the isolates active *in vitro* were also active in soil conditions. Several mechanisms have been proposed for the antibiosis activity, which could be mediated by the secretion of specific or non-specific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds or other toxic substances (Fravel 1988). Hajlaou et al. (1994) reported that such behavior was particularly interesting for biocontrol agents, because the compounds mediating the antibiosis could diffuse in the soil, and direct contact between antagonistic actinomycete and soil-borne pathogen was not necessary.

In addition, the treatment of tomato seeds with TMTD significantly reduced ($P < 0.05$) the disease incidence by over 75% and the rate of healthy seedlings reached 85.3% (Fig. 1A). However, beside this efficacy, the widespread use of chemical fungicides against *R. solani* disease raises persistent problems such as resistance to the fungicide and environment contamination (Grissbuhler et al. 1982).

Among the isolates tested, the *Streptomyces* spp. CA-2 and AA-2 presented the same, non-significant differences at $P < 0.05$ and the same effect in reducing *R. solani* damping-off incidence as the chemical control agent in both sterilized and non-sterilized soils. Coating of tomato seeds by spores of these two isolates allowed the highest rates of healthy seedlings (86.6% and 85.3%, respectively). Furthermore, they significantly increased the seedling fresh weight, seedling length and root length compared to the positive control. Several mechanisms could be involved in plant growth promotion by endophytic actinomycetes. The production of plant growth regulators, such as indol-3-acetic acid and gibberellic acid, the inorganic phosphate solubilization, and the nitrogen fixation could be the most important ones. Several studies have reported plant growth promoting activities of endophytic actinomycetes on tomato seedlings (Coa et al. 2005; El-Tarabily et al. 2008; Goudjal et al. 2013). The enhancement of plant growth by the strains *Streptomyces* sp. CA-2 and AA-2 could contribute to the protection of the plant against pathogenic fungi as previously reported with other *Streptomyces* spp. by Xiao et al. (2002).

The transposition of the ability of the strains CA-2 and AA-2 to colonize the tomato roots demonstrated that their endophytic property was maintained. As defined by Wilson (1995), endophytic actinomycetes, for all or part of their life cycle, invade the tissue of living plants and cause unapparent, asymptomatic infections entirely within plant tissue, but cause no symptoms of disease. A correlation has been shown between the efficacy of biocontrol microorganisms against soil-borne pathogens and their ability to colonize the root system of the plant to be protected, especially when the mode of action used by these bacterial strains is antibiosis or competition for niches and nutrients (Chin-A-Woeng et al. 1998).

The growth promoting effect of the two isolates of actinomycetes seemed to be correlated with root enhancement and shoot production. In the same cases, where the strains were strictly endophytic, such effects were generally attributed to PGRs production (Shi et al. 2009). El-Tarabily et al. (2008) reported that the involvement of PGRs could not only help the seedlings to grow better but could also help the host to compensate for tissue damage caused by the pathogen agent. Several endophytic bacteria have been reported to produce PGRs *in vitro* and to promote the growth of seedlings (Kuklinsky-Sobral et al. 2004; Goudjal et al. 2013). Furthermore, the growth promoting effect is widely attributed to other factors, such as siderophore production and phosphate solubilization (Hamdali et al. 2008) and nitrogen fixation (Ribbe et al. 1997).

The strains CA-2 and AA-2 were related to *S. mutabilis* and *S. cyaneofuscatus*, respectively. Several studies have reported the role of *Streptomyces* strains as potential agents in the biocontrol of soil-borne pathogenic fungi and in promoting plant growth (Lahdenperä et al. 1991; Coa et al. 2004; Dhanasekaran et al. 2005; El-Tarabily et al. 2008). However, this is the first report showing the

same properties in endophytic *Streptomyces* isolated from healthy native plants of the Algerian Sahara.

This study demonstrates the potential of strains *Streptomyces* sp. CA-2 and AA-2 isolated from the roots of such plants in the biocontrol of *R. solani* damping-off and in plant growth promotion for tomato seedlings. It suggests that these *Streptomyces* isolates might have effective antagonistic activities against soil-borne fungi and play a role in promoting the growth of their original native plant hosts. This would contribute to the establishment of the plants and explain their natural seed regeneration under hostile conditions.

Acknowledgements

We thank Dr. Ahmed Boutmedjet of the soil science laboratory, Agronomy department, Laghouat University (Algeria) for his kind determination of the physicochemical properties of the soil used in this study.

References

Altier NA, Thies JA. Identification of resistance to *Pythium* seedling disease in alfalfa using a culture plate method. Plant Dis 1995;79:341–6.

Becker B, Lechevalier MP, Gordon RE, Lechevalier HA. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. J Appl Microbiol 1964;12:421–3.

Boubetra D, Sabaou N, Zitouni A, Bijani C, Lebrihi A, Mathieu F. Taxonomy and characterization of new antibiotics produced by *Saccharothrix* SA198 isolated from a Saharan soil. Microbiol Res 2013;168:223–30.

Chin-A-Woeng TFC, Bloemberg GV, Van der Bij AJ, Van der Drift KMGM, Schripsema J, Kroon B, et al. Biocontrol by phenazine-1- carboxamide producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Mol Plant–Microb Interact 1998;11:1069–77.

Chun J, Bae KS, Moon EY, Jung SO, Lee HK, kim SJ. *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete isolated from a saltern. Int J Syst Evol Micr 2000;50:1909–13.

Chung WC, Huang JW, Huang HC. Formulation of a soil biofungicide for control of damping-off of Chinese cabbage (*Brassica chinensis*) caused by *Rhizoctonia solani*. Biol Control 2005;32:287–94.

Coa L, Qiu Z, You J, Tan H, Zhou S. Isolation and characterization of endophytic *Streptomyces* from surface-sterilized tomato (*Lycopersicon esculentum*) roots. Lett Appl Microbiol 2004;39:425–30.

Coa L, Qiu Z, You J, Tan H, Zhou S. Isolation and characterization of endophytic streptomycetes antagonistis of *Fusarium* wilt pathogen from surface-sterilized banana roots. FEMS Microbiol Lett 2005;247:147–52.

Dhanasekaran D, Sivamani P, Panneerselvam A, Thajuddin N, Rajakumar G, Selvamani S. Biological control of tomato seedling damping off with *Streptomyces* sp. Plant Pathol J 2005;4:91–5.

El-Tarabily KA, Nasser AH, Hardy GE, Sivaithamparam K. Plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes. J Appl Microbiol 2008;106:13–26.

Errakhi R, Bouton F, Lebrihi A, Barakate M. Evidence of biological control capacities of *Streptomyces* spp. against *Sclerotium rolfsii* responsible for damping-off disease in sugar beet (*Beta vulgaris* L.). World J Microbiol Biotechnol 2007;23:1503–9.

Fravel DR. Role of antibiosis in the biocontrol of plant diseases. Annu Rev Phytopathol 1988;26:75–91.

Gees R, Coffe MD. Evaluation of a strain of *Myrothecium raridum* as a potential biological agent against *Phytophthora cinnamomi*. Phytopathology 1989;79:1079–84.

Goodfellow M, Simpson KE. Ecology of streptomycetes. Frontiers Appl Microbiol 1987;2:97–125.

Gopalakrishnan S, Pande S, Sharma M, Humayun P, Keerthi Kiran BK, Sandeep D, et al. Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of *Fusarium* wilt of chickpea. Crop Prot 2011;30:1070–8.

Goudjal Y, Toumatia O, Sabaou N, Barakate M, Mathieu F, Zitouni A. Endophytic actinomycetes from spontaneous plants of Algerian Sahara: indole-3-acetic acid production and tomato plants growth promoting activity. World J Microbiol Biotechnol 2013., http://dx.doi.org/10.1007/s11274-013-1344-y.

Grissbuhler H, Brenneisen P, Fisher HP. Frontiers in crop production: chemical research objectives. Science 1982;217:505–10.

Hajlaou MR, Traquair JA, Jarvis WR, Belanger RR. Antifungal activity of extracellular metabolites produced by *Sporothrix flocculosa*. Biocontrol Sci Technol 1994;4:229–37.

Hamdali H, Hafidi M, Virolle MJ, Ouhdouch Y. Growth promotion and protection against damping-off of wheat by two rock phosphate solubilizing actinomycetes in a P-deficient soil under greenhouse conditions. Appl Soil Ecol 2008;40:510–7.

Hayakawa M, Nonomura H. Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. J Ferment Technol 1987;65:501–9.

Huang X, Zhang N, Yong X, Yang X, Shen Q. Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with *Bacillus pumilus* SQR-N43. Microbiol Res 2011;167:135–43.

Ilic SB, Konstantinovic SS, Todorovic ZB, Lazic ML, Veljkovic VB, Jokovic N, et al. antimicrobial activity of the bioactive metabolites in streptomycete isolates. Microbiology 2007;76:421–8.

Kämpfer P. Genus I. *Streptomyces* Waksman and Henrici 1943. In: Goodfellow, et al., editors. Bergey's manual of systematic bacteriology, Vol. 5. New York Dordrecht, Heidelberg, London: Springer; 2012. p. 1455–767.

Kennedy AC. Bacterial diversity in agroecoystems agriculture. Ecosyst Environ 1999;74:65–76.

Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 2004;6:1244–51.

Kunoh H. Endophytic actinomycetes: attractive biocontrol agents. J Gen Plant Pathol 2002;68:249–52.

Lahdenperä ML, Simon E, Uoti J. Mycostop – a novel biofungicide based on *Streptomyces* bacteria. In: Beemster ABR, Bollen GJ, Gerlagh M, Ruissen MA, Schippers B, Tempel A, editors. Biotic interactions and soil-borne disease. Amsterdam: Elsevier; 1991. p. 258–63.

Liu D, Coloe S, Baird R, Pedersen J. Rapid mini-preparation of fungal DNA for PCR. J Clin Microbiol 2000;38:471.

Meschke H, Schrempf H. *Streptomyces lividans* inhibits the proliferation of the fungus *Verticillium dahliae* on seeds and roots of *Arabidopsis thaliana*. Microb Biotechnol 2010;3:428–43.

Misaghi IJ, Donndelinger IJ. Endophytic bacteria in symptom-free cotton plants. Phytopathology 1990;80:808–11.

Moussa TAA. Studies on biological control of sugar beet pathogen *Rhizoctonia solani* Kühn. Online J Biol Sci 2002;2:800–4.

Parmeter JR, Sherwood RT, Platt WD. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 1969;59:1270–8.

Patil HJ, Srivastava AK, Kumar S, Chaudhari BL, Arora DK. Selective isolation, evaluation and characterization of antagonistic actinomycetes against *Rhizoctonia solani*. World J Microbiol Biotechnol 2010;26:2163–70.

Pouget M. Les relations sol-végétation dans la steppe sud-algéroise. Paris: Orstom; 1980. p. 569.

Ribbe M, Gadkari D, Meyer O. N_2 fixation by *Streptomyces thermoautotrophicus* involves a molybdenum-dinitrogenase and a manganese-superoxide oxidoreductase that couple N_2 reduction to the oxidation of superoxide produced from O_2 by a molybdenum-CO dehydrogenase. J Biol Chem 1997;272:26627–33.

Sabaou N, Bounaga N. Actinomycètes parasites de champignons: étude des espèces, spécificité de l'action parasitaire au genre *Fusarium* et antagonisme dans le sol envers *Fusarium oxysporum* f. sp. *albedinis* (Killian et Maire) Gordon. Can J Microbiol 1987;33:445–51.

Sadeghi A, Hessan AR, Askari H, Aghighi S, Shahidi Bonjar GH. Biological control potential of two *Streptomyces* isolates on *Rhizoctonia solani*, the causal agent of damping-off of sugar beet. Pak J Biol Sci 2006;9:904–10.

Schulz B, Wanke U, Draeger S, Aust HJ. Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. Mycol Res 1993;97:1447–50.

Shahidi Bonjar GH, Barkhordar B, Pakgohar N, Aghighi S, Biglary S, Rashid Farrokhi P, et al. Biological control of *Phytophthora drechsleri* Tucker, the causal agent of pistachio gummosis, under greenhouse conditions by use of actinomycetes. Plant Pathol J 2006;5:20–3.

Shen D. Microbial diversity and application of microbial products for agricultural purposes in China agriculture. Ecosyst Environ 1997;62:237–45.

Shi Y, Lou K, Li C. Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. Biol Fertil Soils 2009;45:645–53.

Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 1966;16:313–40.

Suzuki S, Yamamoto K, Okuda T, Nishio M, Nakanishi N, Komatsubara S. Selective isolation and distribution of *Actinomadura rugatobispora* strains in soil. Actinomycetologica 2000;14:27–33.

Taechowisian T, Peberdy JF, Lumyong S. Isolation of endophytic actinomycetes from selected plants and their antifungal activity. World J Microbiol Biotechnol 2003;19:381–5.

Trejo-Estrada SR, Sepulveda IR, Crawford DL. In vitro and in vivo antagonism of *Streptomyces violaceusniger* YCED9 against fungal pathogens of turfgrass. World J Microbiol Biotechnol 1998;21:81–90.

Wilson D. Endophyte – the evolution of a term and clarification of its use and definition. Oikos 1995;73:274–6.

Xiao K, Kinkel LL, Samac DA. Biological control of *Phytophthora* root rots on alfalfa and soybean with *Streptomyces*. Biol Control 2002;23:285–95.