Characterization and antagonistic properties of *Streptomyces* strains isolated from Saharan soils, and evaluation of their ability to control seedling blight of barley caused by *Fusarium culmorum*

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Significance and Impact of the Study: The genus *Fusarium* is considered to be one of the most problematic phytopathogenic fungi for crop culture worldwide. Inside this genus, *F. culmorum* is the aetiological agent of seedling blight in various monocotyledonous plants such as barley and cause extensive yield and quality losses in humid and semi-humid regions. Biological control may be a successful alternative to chemical control, particularly with the controversy surrounding the use of the fungicides and the limited obtained results to control *F. culmorum*. This study highlights the effectiveness of some antagonistic *Streptomyces* isolated from Algerian Saharan soils to control *F. culmorum* by the reduction in disease occurrence and disease severity suggesting their use on microbial biocontrol formulation against soilborne diseases.

Keywords
actinomycetes, biocontrol, *Fusarium culmorum*, seedling blight, *Streptomyces*, taxonomy.

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Abstract

During a screening for potential plant disease control actinomycetes, a total of 133 strains were isolated from Saharan soil samples of seven Algerian regions by dilution technique on chitin-vitamins agar medium. Screening for antagonistic properties using streak assay method showed that 25% of isolates demonstrated strong activities against a wide range of plant pathogenic fungi. Due to their strong anti-*Fusarium* activities, six of these isolates were selected and subsequently related to *Streptomyces* species by polyphasic analysis. These isolates were evaluated for their biocontrol ability against *Fusarium culmorum*, a serious pathogenic fungus of cereals crops related to damping-off and seedling blight resulting in yield loss. Barley seeds were chosen as cereal plant model. Surface bacterized seeds with TW3, RI3 and TW2 strains expressed the highest performances and permit to reduce significantly both the disease occurrence on seedlings (62–76%) and the extent of seedling blight symptoms (over than 95%). However, a negative effect on plant establishment was observed for RI3 treatment.

Introduction

Actinomycetes are a group of filamentous bacteria widely spread in nature and may occur in extreme habitats such as arid Saharan soils. Great interests are given to these micro-organisms because of their ability to provide broad range of bioactive compounds with potential benefit in human health, industrial process and agriculture (Lazzarini *et al.* 2000). In Algeria, microecological studies of Saharan soils have demonstrated appreciable actinomycetes biodiversity (Sabaou *et al.* 1998), which permitted to find out novel species and antibiotics (Zitouni *et al.* 2004; Boudjella *et al.* 2010; Merrouche *et al.* 2010).

Approximately, two-third of natural antibiotics were isolated from actinomycetes and about 75% of antibiotic producing micro-organisms belong to the genus *Streptomyces* (Newman *et al.* 2003). Therefore, antagonistic impacts of *Streptomyces* on pathogenic fungi were deeply investigated, but few species have been implicated in biocontrol of plant disease (Bressan 2003).

In the recent years, search for alternatives to chemical control of plant pathogens, such as biological control, gained great interest due to the emergence of fungicide-resistant pathogens, irreversible environmental depletion and health concerns for both producers and consumers (Compant *et al.* 2005). Furthermore, complex life cycle and soil-persistence of several important crop pathogens notably limit effectiveness of fungicide to control soil-borne pathogens (Wagacha and Muthomi 2007). *Fusarium culmorum* is one of these problematic pathogens, source of serious damages on field crops all over the world, particularly on wheat and barley (Wagacha and Muthomi 2007). *Fusarium culmorum* cause several devastating diseases with seed and soilborne origin occurring at all stage of plant development: seedling blight, root rot, foot rot and head blight (scab) (Fernandez and Jefferson 2004). Damages caused by the fungus are expressed in a loss of germinability, a reduced emergence, a postemergence blight of seedlings and a reduction in grain quality by mycotoxins contamination (trichothecenes and fusarins) (Demeke *et al.* 2005), which finally result in extensive yield and quality losses to cereals in humid and semi-humid regions. In USA alone, the total economic impacts were amounted to millions of dollars per year and have been estimated at $6·2 billion (USD) from 1993 to 2001 (Nganje *et al.* 2004). In this context, use of potentially antagonistic bacteria applied as seed treatment (bacterization) could be a promising approach to control *F. culmorum* and reduce its impact. Thus, numerous studies with bacteria and fungi, including *Bacillus*, *Pseudomonas* and *Trichoderma*, were used as seed treatment but their effectiveness to suppress *F. culmorum* seedling blight were not clearly established (Johansson *et al.* 2003; Amein *et al.* 2008). Because of their great ability to produce many active substances, members of the genus *Streptomyces* appear as a prospective alternative to control *F. culmorum*.

Through this way, this study aimed to select isolates of *Streptomyces* from Saharan soils of Algeria and estimate their effectiveness to control *F. culmorum* seedling blight of barley. Antagonistic abilities of isolates were evaluated towards a range of plant pathogenic fungi. The capacity of antagonistic strains to reduce seedling blight symptoms and disease impacts were then assessed. Diversity study of selected isolates was also carried out and permits taxonomic and phylogenetic characterization.

Results and discussion

Antagonistic properties of actinomycete isolates

A total of 133 isolates were collected from 10 analysed soils samples of seven locations: Adrar (23 isolates), Bechar (12 isolates), Beni Abbes (25 isolates), El Oued (10 isolates), Ghardaia (22 isolates), Ouargla (14 isolates) and Tamanrasset (27 isolates).

Among isolates, 33 (25%) showed a strong antagonistic activity against the 14 phytopathogenic and/or toxigenic fungi tested (Table 1). Six isolates (noted AD100, AD107, AD109, TW2, TW3 and RI3) exhibited the strongest *in vitro* antifungal activities, especially with regard to their significant anti-*Fusarium* activities. The most sensitive fungi were *F. culmorum*, *Fusarium oxysporum* f. sp. *lini*, *F. oxysporum* f. sp. *albedinis*, *Fusarium equiseti and Penicillium glabrum*. The less sensitive fungi were *Fusarium moniliforme*, *Fusarium sporotrichoides*, *Fusarium graminearum*, *Fusarium proliferatum*, *Aspergillus flavus*, *Aspergillus parasiticus* and *Penicillium expansum*. Furthermore, only few isolates strongly inhibit *Aspergillus carbonarius* and *Aspergillus niger*, such as AD100, AD109 and TW2.

The antagonistic potential of actinomycetes from Algerian desert towards plant pathogenic fungi were already reported and involved to the production of extracellular hydrolytic enzymes (Sabaou and Bounaga 1987) and moreover secondary antifungal compounds (Boudjella *et al.* 2010; Merrouche *et al.* 2010), but no recent studies interested in value of these antagonistic properties for biocontrol purpose. Trejo-Estrada *et al.* (1998) demonstrated a clear correlation between the ability of *Streptomyces violaceusniger* YCED9 to produce antibiotic compounds in soil and its efficiency to control plant pathogens belonging to *Fusarium* genus. Therefore, in the following sections of the survey, we focused on characterization and biocontrol evaluation for the six most significant strains AD100, AD107, AD109, TW2, TW3 and RI3.

Taxonomy of antagonistic actinomycetes

The six isolates grew well on all media used. They formed nonfragmented and colourless substrate mycelium. The aerial mycelium was greenish grey for all isolates and produced numerous nonmobile and spiralled spores chains on sporophores. Diffusible pigments were not observed (Table 2). All strains contained LL-diaminopimelic acid isomer and glycine in cell wall. The whole-cell hydrolysates contained galactose and glucose for isolates AD107, AD109, TW2 and TW3, and galactose, glucose and ribose for AD100 and RI3. Thus, it indicates that the six isolates have cell wall chemotype IC (Lechevalier and Lechevalier 1970). The phospholipid pattern was type PII (Lechevalier *et al.* 1977) characterized by the presence of phosphatidylethanolamine. Based on the morphological and chemical characterization, the six isolates were classified as a member of the genus *Streptomyces* (Holt *et al.* 1994).

Physiologically, the six isolates can be separated into two groups. The first group, including AD100, AD107

Table 1 Antagonistic properties of actinomycetes isolated from Saharan soils

Origin	Actinomycetes strains	Activity* (mm) against													
		Fc	*Fm*	*Fs*	*Fg*	*Fol*	*Foa*	*Fp*	*Fe*	*Ac*	*An*	*Af*	*Ap*	*Pg*	*Pe*
Adrar	AD100	21	5	0	3	40	40	2	27	35	34	8	6	22	2
	AD107	20	8	2	4	26	20	5	20	10	16	1	7	25	2
	AD109	9	2	0	0	17	22	10	20	25	26	2	4	25	3
	AD115	0	7	1	3	0	0	0	0	9	5	1	0	5	5
	AD119	0	2	0	0	0	0	0	0	8	2	0	0	0	4
	AD125	1	1	4	3	1	1	0	0	8	6	3	0	9	2
Beni Abbes	BA102	1	3	8	6	0	1	0	0	1	2	4	0	0	5
	BA106	0	0	0	0	0	0	0	0	10	3	1	0	0	4
	BA117	0	0	0	0	0	0	0	1	19	4	0	0	10	10
	BA118	0	0	0	6	0	0	0	0	7	0	1	0	0	4
	BA120	0	0	0	1	0	0	2	1	1	3	5	0	2	0
	BA121	0	5	2	0	0	0	3	0	2	5	2	5	4	0
	BA122	6	0	1	5	0	0	0	1	26	5	2	0	5	2
	BA123	0	0	0	6	6	3	0	0	10	2	5	3	5	7
	BA125	0	0	0	1	0	0	0	0	6	0	0	0	0	2
Ouargla	DP10	2	2	6	7	1	7	0	0	2	10	0	0	0	0
	DP13	0	1	0	5	0	7	0	0	8	9	0	0	0	0
	DP9	6	4	3	5	6	9	4	8	2	2	0	0	20	0
Tamanrasset	HG100	0	2	2	0	0	0	0	0	10	5	0	0	9	5
	HG101	0	1	0	3	0	0	3	0	1	1	1	6	1	3
	HG105	0	4	2	0	0	0	0	0	10	3	0	3	0	1
	HG107	0	3	3	7	3	22	9	1	12	11	0	0	0	2
	HG118	0	6	0	0	0	0	0	0	8	7	0	0	7	2
	HG121	0	7	0	0	2	0	0	0	9	10	0	2	6	10
Ghardaia	RI3	35	3	0	0	16	29	0	34	3	14	2	5	30	1
	RI7	5	0	0	0	17	5	1	1	14	9	3	0	1	0
	RI8	0	0	0	5	0	0	0	0	9	9	2	0	0	3
El Oued	TW2	26	7	5	5	16	33	2	40	29	11	3	5	30	4
	TW3	33	2	2	2	25	26	5	28	2	1	2	0	23	1
	TW10	6	0	1	1	1	0	1	1	10	5	2	1	4	7
Bechar	WAB3	0	0	0	2	4	6	2	13	2	1	2	0	4	2
	WAB6	6	0	0	1	0	0	4	7	14	13	0	0	2	1
	WAB10	0	2	0	10	1	9	1	15	1	2	0	0	15	2

Fc, Fusarium culmorum; Fm, Fusarium moniliforme; Fs, Fusarium sporotrichoides; Fg, Fusarium graminearum; Fol, Fusarium oxysporum f. sp. *lini; Foa, Fusarium oxysporum* f. sp. *albedinis; Fp, Fusarium proliferatum; Fe, Fusarium equiseti; Ac, Aspergillus carbonarius*; *An, Aspergillus niger*; *Af*, *Aspergillus flavus*; *Ap, Aspergillus parasiticus*; *Pg, Penicillium glabrum*; *Pe, Penicillium expansum*.

For the other 100 strains of actinomycetes, activities were no existent or very weak ($\leq$2 mm).

*Activity estimated by measuring the length of inhibition between actinomycetes and target micro-organisms.

and AD109 (similar to each other), differs from the second group, including TW2, TW3 and RI3 (also similar to each other) by the use of sucrose and xanthine, nitrate reduction and growth in the presence of 10% NaCl, but not at 45°C (Table 2).

The complete 16S rDNA sequence (1462–1466 bp) of AD100, AD107, AD109, TW2, TW3 and RI3 were determined and deposited in the GenBank data library under the accessions numbers JQ687121–JQ687126, respectively. The sequences were aligned with those *Streptomyces* reference species available in the GenBank database, which confirmed that these six strains belong to *Streptomyces* genus. The illustrated phylogenetic tree (Fig. 1) shows the distribution of the six strains in two clusters. The first cluster, formed by AD100, AD107 and AD109, was most closely related to the species *Streptomyces atrovirens* NRRL B-16357^T (similarity level ranged from 99·7 to 99·8%); the second cluster, formed by TW2, TW3 and RI3, was most closely related to the species *S. gancidicus* NBRC 15412^T and *Streptomyces pseudogriseolus* NBRC 12902^T. For both strains TW2 and RI3, similarity level was 99·6%, while TW3 showed only 99·2% similarity with the two species of S*treptomyces*. Phenotypically, the strains AD100, AD107 and AD109 were found to resemble to *S. atrovirens* and the strains TW2, TW3 and RI3 resemble to *S. gancidicus* and *S. pseudogriseolus* (Locci 1989).

Table 2 Phenotypic characteristics of selected antagonistic actinomycetes

Properties	Actinomycetes isolates					
	AD100	AD107	AD109	TW2	TW3	RI3
Morphological characteristics						
Spore chain morphology	S	S	S	S to S.RA.RF	S to S.RA.RF	S to S.RA.RF
Colour of aerial mycelium	Greenish grey	Greenish grey	Greenish grey	Greenish grey	Greenish grey	Greenish grey
Colour of substrate mycelium	Colourless to pale yellow	Colourless	Colourless to pale brown	Colourless	Colourless to yellow brown	Colourless
Diffusible pigment	−	−	−	−	Brownish yellow	−
Melanoid pigments	−	−	−	−	−	−
Carbon source utilization						
Arabinose	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Inositol	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+
Melibiose	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−
Rhamnose	+	+	+	+	+	+
Salicine	+	+	+	+	+	+
Sucrose	+	+	+	−	−	−
Xylose	+	+	+	+	+	+
Nitrogen source utilization						
Histidine	+	+	+	+	+	+
Phenylalanine	+	+	+	+	+	+
Proline	+	+	+	+	+	+
Hydrolysis of						
Starch	+	+	+	+	+	+
Xanthine	+	+	+	−	−	−
Nitrate reduction	+	+	+	−	−	−
Growth in the presence of						
NaCl 10% w/v	+	+	+	−	−	−
Sodium azide 0·01% w/v	−	−	−	−	−	−
Phenol 0·1% w/v	+	+	+	+	+	+
Penicillin 10 UI	+	+	+	+	+	+
Rifampicin 50 μg ml^{-1}	−	−	−	−	−	−
Growth at 45°C	−	−	−	+	+	+

+, positive; −, negative; S, spiral; RA, retinaculiaperti; RF, flexuous.

However, as the similarity percentage of the 16S rDNA between our strains and these species is >97%, DNA–DNA hybridization is necessary in this case for a final decision on their taxonomy.

However, Chugasova *et al.* (1975) found that *S. atrovirens* produces bioactive diffusible pigments, while strains AD100, AD107 and AD109, which were closely related to this species, did not produce any pigment. On the other hand, Zhang *et al.* (2004) isolated an antagonistic strain of *S. pseudogriseolus* with antibacterial and antitumoural activities. Furthermore, Aiso *et al.* (1956) have identified in *S. gancidicus* an antibiotic, named gancidin, with antitumoural and antifungal properties. However, the strains TW2, TW3 and RI3, which were closely related to *S. pseudogriseolus* and *S. gancidicus*, exhibited both antibacterial and antifungal activities.

Biocontrol assay of antagonistic actinomycetes

To evaluate the biocontrol potential of the selected six strains, we focused on *F. culmorum*, a serious pathogenic fungus of cereals crops related to damping-off and seedling blight resulting in yield loss. The results concerning the effects of antagonistic actinomycete strains on seedling blight biocontrol experiment are given in Table 3.

Untreated plants (control) of barley exhibited no disease symptoms and appeared healthy (95·6% of emerged plants), whereas pre-infested seeds (noted FC) showed a high disease impact (17·4% plant emergence reducing and 70% plants diseased). Compared with FC, pre-infested and treated seeds with one of selected strains (noted FC-AD100, FC-AD107, FC-AD109, FC-TW2 and FC-TW3) did not exhibit any significant improving on plant

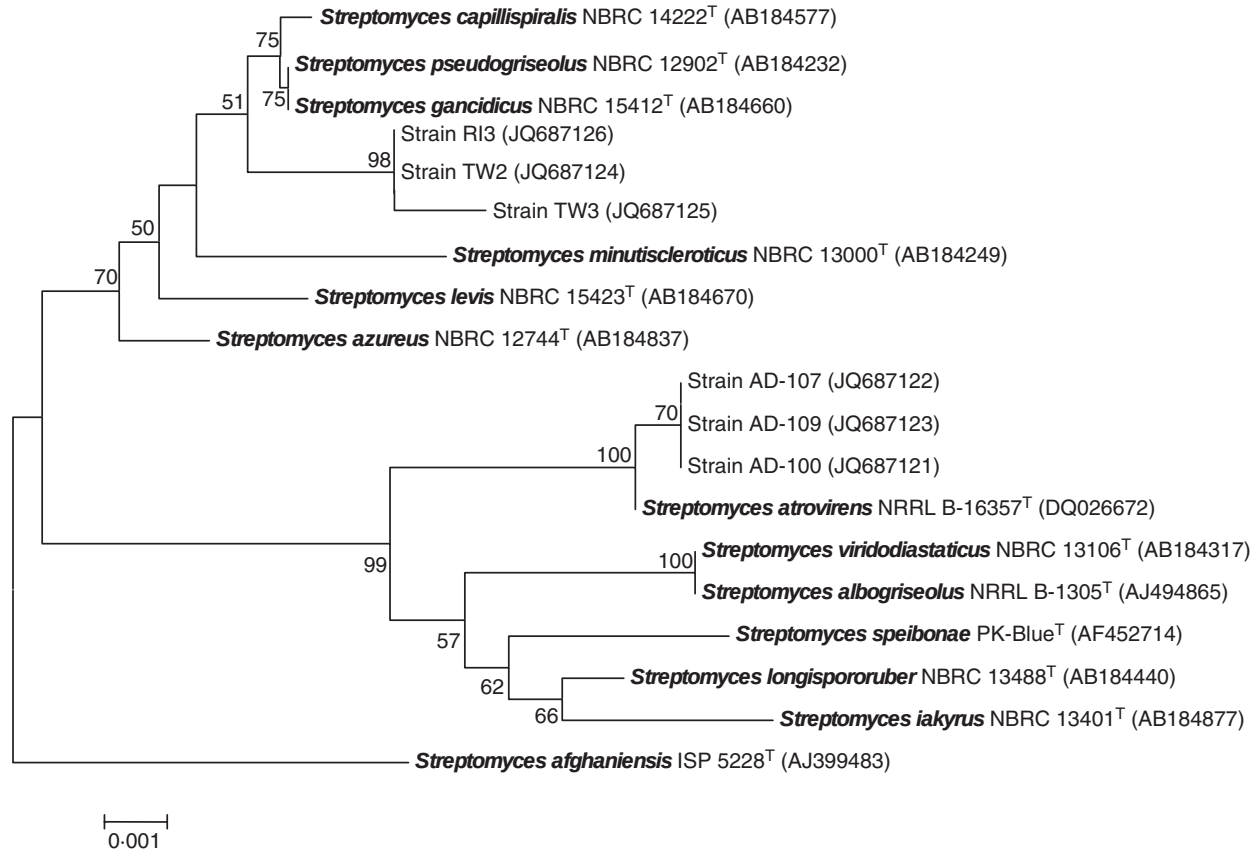

Figure 1 Neighbour-joining tree based on 16S rDNA sequences showing the relations between strains AD100, AD107, AD109, TW2, TW3 and RI3 and type-species of the genus *Streptomyces*. The numbers at the nodes indicate the levels of bootstrap support based on neighbour-joining analyses of 1000 resampled data sets. Bar, 0·001 nt substitution per nt position. *Streptomyces afghaniensis* ISP 5228^T has been used as outgroup.

Table 3 Effects of antagonistic actinomycetes strains on seedling blight biocontrol experiment

	% Plants	
Treatment*	Emerged	Diseased
Control	95·7^b	0^a
FC	78·3^{ab}	70·0^c
FC-AD100	90·0^{ab}	65·0^c
FC-AD107	86·7^{ab}	62·7^c
FC-AD109	83·3^{ab}	57·4^{bc}
FC-TW2	85·0^{ab}	26·7^{ab}
FC-TW3	78·0^{ab}	16·8^a
FC-RI3	60·0^a	24·4^{ab}

Means with the same letter in the same column are not significantly different at $P = 0{\cdot}05$.

*Seeds were co-inoculated with *Fusarium culmorum* (FC) and one of the selected actinomycetes strains (AD100, AD107, AD109, TW2, TW3 or RI3).

emergence. However, pre-infested seeds treated with RI3 (FC-RI3) played a negative effect on plant establishment: 18·3 and 35·7% more reducing of plant emergence than FC and control, respectively. Same negative effect was recorded when healthy seeds (no infested) were treated with RI3 (only 63·2% seeds emerged with more than 30% reduction in seedling weight and length compared with control). This deleterious effect was already recorded and related to the ability of some *Streptomyces* to produce phytotoxic metabolites, which were considered as herbicidal compounds (Loria *et al.* 2006; Bignell *et al.* 2010).

Seeds treatment significantly reduced disease occurrence on seedlings (Table 3). TW3, RI3 and TW2 showed the best performances with 76, 65 and 62% disease reduction, respectively, while AD109 treatment induced only 18% decrease in the disease incidence. AD100 and AD107 showed no significant reduce on disease percentage.

Regarding to the disease severity (Fig. 2), all strains significantly reduced *F. culmorum* seedling blight symptoms. The effectiveness of TW3, RI3 and TW2 treatments to reduce disease severity were expressed by low severity scores (0·4, 0·8 and 0·9, respectively), while AD100, AD107 and AD109 were the less efficient to reduce disease symptoms (8·91, 7·04 and 8·38, respectively).

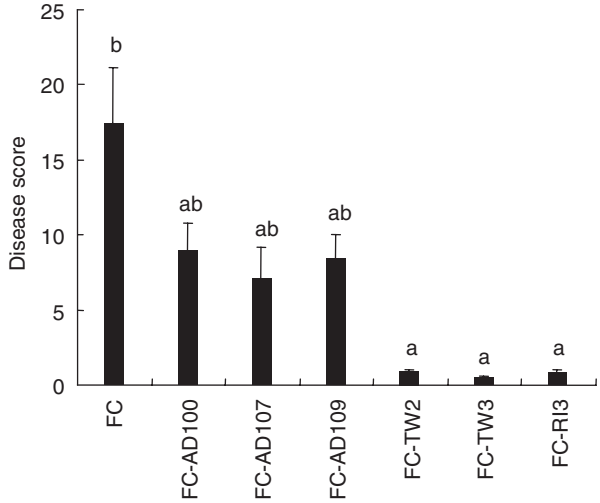

Figure 2 Disease symptom development on barley variety Magenta. Seeds were coinoculated with *Fusarium culmorum* (FC) and one of the selected actinomycetes strains (AD100, AD107, AD109, TW2, TW3 or RI3). Bars indicate standard error of the mean; columns with the same letters are not significantly different at $P = 0{\cdot}05$.

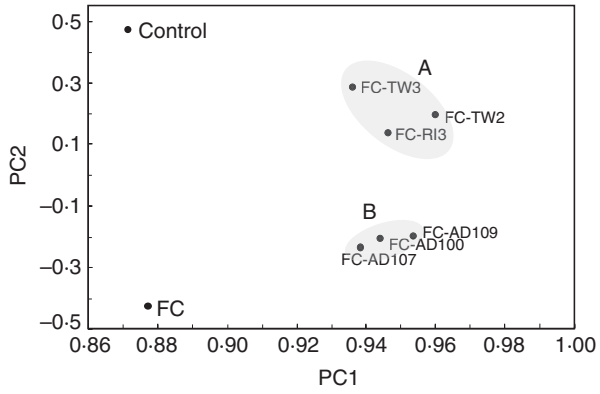

Figure 3 Principal component (PC) analysis plot (PC1 *vs* PC2) obtained from data on biocontrol assay of tested strains against *Fusarium culmorum* seedling blight. Groups have been indicated by grey background.

The principal component (PC) analysis from all data recorded on the biocontrol assay (Fig. 3) provided a general view of biocontrol efficiency for tested strains against *F. culmorum* seedling blight. Together, the first axis (PC1) and second axis (PC2) explained 94% of the differences between treatments (86% for PC1 and 8% for PC2). As suggested by ANOVAS results, PC analysis clearly split up strains in two groups: first group (A), constituted with FC-TW2, FC-TW3 and FC-RI3 and second group (B), constituted with FC-AD100, FC-AD107 and FC-AD109. Group A strains were characterized by high biocontrol performances and group B strains were characterized by low performances. Among strains of group A, TW3 treatment exhibited the closest PC values to control, which express the best biocontrol performance. Relative weak PC values of RI3 showed the significant impact of its deleterious effects (Table 3) on the global biocontrol efficiency decrease. Thus, RI3 appeared to be unsuitable for biocontrol purpose.

Hence, groups given by PC analysis corresponds to the two clusters shown by the phylogenic study, where group A strains were related to *S. gancidicus* NBRC 15412^{T} and *S. pseudogriseolus* NBRC 12902^{T}, and group B strains related to *S. atrovirens*. In the other hand, among strains of group A, the most effective strain (TW3) was the less closer to these two species of *Streptomyces*.

Actinomycetes, particularly those belonging to the genus *Streptomyces*, are known for the production of several antibiotics and some strains are used in the biological control of plant diseases caused by many phytopathogenic fungi, including *Alternaria brassicola*, *Botrytis cinerea*, *Fusarium avenaceum*, *F. culmorum*, *F. oxysporum* f. sp. *dianthi*, *Pythium debaryanum*, *Phomopsis sclerotioides*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Tahvonen 1982; Tahvonen and Avikainen 1987). For example, Mycostop^{TM} is a biofungicide that contains *Streptomyces griseoviridis* as the active ingredient. This product is available in United States and Europe (Doumbou *et al.* 2001). Several properties associated with actinomycetes might explain the ability of several of them to act as biocontrol tools. Those properties are the ability to colonize plant surface, the antibiosis against plant pathogens, the synthesis of particular extracellular proteins and the degradation of phytotoxins (Doumbou *et al.* 2001).

Our biocontrol assay highlights effectiveness of some active Saharan soilborne *Streptomyces* to control *F. culmorum* seedling blight when used as seed treatment. This result confirms previous studies which demonstrated potential use of some Saharan *Streptomyces* strains as biocontrol agents (Errakhi *et al.* 2007; Loqman *et al.* 2009). In fact, there was significant correlation between *in vitro* antagonistic properties and the reduction in both disease occurrence ($r = 0{\cdot}81$, $P \leq 0{\cdot}05$) and disease severity ($r = 0{\cdot}85$, $P \leq 0{\cdot}05$).

Further investigations need to be performed to confirm consistence of biocontrol effectiveness of strain TW3 and TW2 in field conditions focusing on micro-organism establishment and maintenance in plant and/or rhizosphere.

Materials and methods

Origin and isolation of actinomycetes

Thirteen nonrhizospheric soil samples (5–20 cm of depth) from Saharan regions of Algeria were collected at Adrar

(27°51′N; 0°19′W), Bechar (31°34′N; 2°16′W), Beni Abbes (30°08′N; 2°10′W), El Oued (33°19′N; 6°52′E), Ghardaia (32°24′N; 3°48′E), Ouargla (32°0′N; 5°16′E) and Tamanrasset (22°48′N; 5°27′E). The soil textures were sandy loam to sandy with basic pH (between 8·2 and 8·8), non-salt (electrical conductivity between 0·18 and 0·25 mS cm^{-1}), with a low amount of organic carbon (between 0 and 0·48%).

Actinomycetes strains were isolated by dilution technique on chitin-vitamins agar medium (Hayakawa and Nonomura 1987) supplemented with cycloheximide (50 mg l^{-1}) to avoid invasive fungal development. The dry soil sample was suspended in sterile distilled water and diluted. Aliquots (0·2 ml) of each dilution were spread onto the culture medium. The plates were incubated at 30°C for 2 weeks.

Antagonistic properties of actinomycetes

The antagonistic properties of actinomycetes were determined by streak method. Each strain was cultivated in strip shape on ISP-2 medium (Shirling and Gottlieb 1966) plate for 10 days at 30°C, and then targets micro-organisms were seeded in crossed streaks to actinomycetes cultivation. Antagonistic activities scoring were evaluated by measuring inhibition length between actinomycetes strain and the target micro-organism after 48-h incubation at 30°C. The target micro-organisms (Table 1) were phytopathogenic and/or toxigenic filamentous fungi.

Phenotypical and chemical characterization of antagonistic actinomycetes

Cultural and micromorphological characteristics of selected actinomycetes were examined according to the method of Shirling and Gottlieb (1966) on yeast extract/malt extract agar (ISP-2), oatmeal agar (ISP-3), inorganic salt/starch agar (ISP-4) and glycerol/asparagine agar (ISP-5) media at 30°C for 14 days.

For the chemical analysis of cellular constituents, isolates were grown on ISP-2 broth medium for 4 days at 30°C. Mycelia were harvested by centrifugation and then used for chemical analyses of diaminopimelic acid isomer (Becker *et al.* 1964), whole-cell sugar (Lechevalier and Lechevalier 1970) and phospholipids (Minnikin *et al.* 1977).

For the physiological study, 23 tests were considered (Locci 1989), including the utilization of 11 carbohydrate compounds as sole carbon source, the assimilation of three amino acid as sole nitrogen source, the degradation of starch and xanthine, the production of nitrate reductase, the growth in the presence of phenol (0·1%), sodium azide (0·01%), penicillin (10 UI), rifampicin (50 μg ml^{-1}) and NaCl (10%) and the growth at 45°C.

16S rDNA sequencing and phylogenetic analysis of antagonistic actinomycetes

Genomic DNA of strains was extracted for 16S rDNA analysis according to the method of Liu *et al.* (2000), using the forward FC27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse RC1492 primers (5′-GGTTACCTTGTTACGACTT-3′). Then, the products were purified and sequenced. The similarities of the 16S rRNA gene sequences between strains were calculated on the basis of pairwise alignment using the EzTaxon-e server (Kim *et al.* 2012). Phylogenetic and molecular evolutionary analyses were carried out using MEGA version 5.0 (Tamura *et al.* 2011) package. The 16S rRNA gene sequences of actinomycetes strains were aligned against neighbouring nucleotide sequences using the CLUSTAL W program (Thompson *et al.* 1994). Phylogenetic tree was constructed by using the neighbour-joining method (Saitou and Nei 1987) with Jukes and Cantor (1969) model. Bootstrap analysis (Felsenstein 1985) was performed to evaluate the reliability of the tree topology.

Assay for antagonistic properties of actinomycete isolates

Seed material. Seeds of barley (*Hordeum distichum* L.) variety Magenta (rustic winter barley) were used in this study.

Fungi and actinomycetes inocula preparation. Pathogenic *F. culmorum* strain isolated from barley fields was obtained from Department of Plant Diseases, High School of Agriculture, Algiers, Algeria. This strain was cultured on potato dextrose agar (potato infusion 200 g, dextrose 20 g, agar 20 g, distilled water 1000 ml, pH 6·0) plates and incubated at 25°C for 7 days. Selected actinomycetes were grown on ISP-2 medium (malt extract 10 g, yeast extract 4 g, glucose 4 g, distilled water 1000 ml, pH 7·2) plates and incubated at 30°C for 10 days to obtain an optimum sporulation. Both *F. culmorum* macroconidia and actinomycetes spores suspensions were obtained by scraping from the culture surface with a glass slide, homogenized in sterile distilled water and filtered through a double layer of sterile gauze. The concentrations were adjusted to 10^5 macroconidia ml^{-1} of *F. culmorum* and 10^8 CFU ml^{-1} for actinomycetes strains by haemocytometre chamber counting method.

Biocontrol assay. Effects of selected actinomycetes on *F. culmorum* seedling blight of barley were assessed using the following method: surface-sterilized seeds (5% NaClO; 0·2% Tween 20; 3 min) were soaked on antagonistic actinomycete spores suspension (10^8 CFU ml^{-1}; 60 min) obtained as described previously and air-dried under laminar flow. Seeds were then put into 9-cm Petri dishes (20 seeds per plate) containing filter paper pre-inoculated

with 3×10^5 macroconidia of *F. culmorum*. Sterile distilled water was used as control treatment. Experiments were conducted in randomized design and repeated at least three times.

Disease severity rating. Visible disease symptoms on stems were scored at 12 days postplanting (70–80% relative humidity; 20 ± 2°C; 13 h of light period) according to the scoring system described by Khan *et al.* (2006). Seedling blight score was the product of lesion length (cm) by lesion colour: lesion colour scale: 0, no disease; 1, very slight brown necrosis; 2, slight/moderate brown necrosis; 3, extensive brown necrosis; and 4, extensive black necrosis.

Data analysis. All biocontrol data were analysed by analysis of variance (ANOVA) and principal component (PC) analysis. Mean separation was accomplished by Newman and Keuls multiple range test. Pearson's correlation coefficients (r) were also calculated. For all data, significance was evaluated at $P \leq 0{\cdot}05$.

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