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Biocontrol of Wheat Fusarium Head Blight (FHB) by *Streptomyces* spp. Isolated from the Rhizosphere of *Astragalus gombo* Coss. & Dur. and *Ononis angustissima* Lam.

[1,2]*Ghadbane Mouloud,* [1]*Medjekal Samir,* [2]*Belhadj Hani and* [2]*Harzallah Daoud*

[1]Department of Natural and Life Sciences, Faculty of Sciences,
University of *Mohamed Boudiaf-M'sila*, P.O. Box: 166 Ichebilia, M'sila, 28000, Algeria
[2]Laboratory of Applied Microbiology, Department of Microbiology,
Faculty of Natural and Life Sciences, University Ferhat Abbas, Setif 1 19000, Algeria

Abstract: *Fusarium culmorum* is a ubiquitous soil-borne fungus able to cause foot and root rot and Fusarium head blight on different small-grain cereals, in particular wheat and barley. Many rhizospheric actinobacteria isolated from *Ononis angustissima* Lam. and *Astragalus gombo* Coss & Dur. were tested for their antagonism against different pathogenic microorganisms *in vitro*. Among 120 tested isolates, only six isolates (ON03, BI26, BI28, OR6, ON02 and BI15) present a potent antagonistic effect against tested bacterial and fungal pathogens. Cultural and chemical characteristic studies strongly suggested that these strains belong to the genus *Streptomyces*. All of the six *Streptomyces* spp. isolates produced relatively high levels of IAA and chitinase. Five *Streptomyces* spp. strains (BI15, BI26, BI28, ON03 and ON02) solubilize phosphate and four strains (BI15, BI26, ON03 and ON02) produced protease. The *in vivo* biocontrol assays revealed that the *Streptomyces* strains significantly promoted the growth of the wheat plants and showed greater suppression of Wheat Fusarium head blight (FHB) disease. All of the six *Streptomyces* spp. strains showing good effect in increasing seedling emergence of wheat. Our results show that the selected *Streptomyces* spp. from rhizosphere of *Ononis angustissima* and *Astragalus gombo* could be an interesting source for antimicrobial bioactive substances and can efficiently protect wheat against FHB disease.

Key words: Antimicrobial activity · Bioactive substances · Chitinase · Indole acetic acid · Phenotypic properties · Wheat foot root rot and Fusarium head blight

INTRODUCTION

Wheat is the most widely grown crop in the world, representing a major renewable resource for food, feed and industrial raw materials [1]. Fusaria have long been recognized as pathogens of many plant species. Wheat and other small grain cereals may be attacked by a wide range of *Fusarium* spp. and on different plant organs. However, infestation of the ears appears to be the most critical, leading to Fusarium head blight (FHB), also known as scab. FHB is a pre-harvest disease, but *Fusarium* species can grow post-harvest if wet grain is not dried efficiently and quickly. *Fusarium graminearum* and *F. culmorum* are the predominant *Fusarium* species infecting wheat [2, 3]. *Fusarium culmorum* belongs to section Discolour, whose other species include *F. graminearum* Schwabe, *F. sambucinum* Fuckel, *F. crookwellense* Burgess, Nelson & Toussoun, *F. heterosporum* Nees and *F. reticulatum* Mont. [2]. The economic impact of *Fusarium* damage is of wheat great importance throughout the world [4, 5].

The actinobacteria represent a well-known and extremely diverse group of Gram-positive, aerobic, filamentous bacteria belonging to the order Actinomycetales. These bacteria are one of the major components of the microbial populations present in soil. They have the capacity to synthesize many different biologically active secondary metabolites such as antimicrobial agents. Bacteria belonging to the genus *Streptomyces* are widely recognized as industrially

Corresponding Author: Ghadbane Mouloud, Department of Natural and Life Sciences, Faculty of Sciences, University of Mohamed Boudiaf-M'sila, P.O. Box: 166 Ichebilia, M'sila, 28000, Algeria.

important microorganisms and produce many agriculturally and medically useful antibiotics [6,7], antifungal [8, 9], anticancer [10, 11], antioxidant [12, 13], insecticides [14], antifeedant agents [15] and herbicides [16], as well as plant growth hormones and enzymes [17, 18]. The search for new antimicrobials has not been limited to the medicinal field, but also extends to crop protection. Development of fungicide-resistant plant pathogens as well as excessive and indiscriminate use of synthetic agrochemicals has led to ecological imbalances in soil and human health [19]. Therefore, the search for alternatives to chemical control of plant pathogens, such as biological control, has gained momentum in recent years. Biological sources for the control of plant diseases remain an important objective for sustainable agricultural practices [20, 21]. The antifungal potential of extracellular metabolites from *Streptomyces* against some fungi was previously reported. Somme *Streptomyces* species are used as biocontrol agents against phytopathogenic fungi [22, 23].

Microorganisms from extreme environments have gained considerable attention in recent years because of its diversity and biological activities, mainly due to its ability to produce novel chemical compounds of high commercial value [13]. Algerian desert soils are exposed to an arid climate and represent particular ecosystems. Previous studies have reported the abundance and diversity as well as secondary metabolites of actinobacteria in these soils [24, 25, 26]. The screening of actinobacteria from diverse rhizosphere of indigenous plants growing in Southern of Algeria (Sahara) deserves special attention to explore the potentialities of the diverse microflora of this region, as Biskra is part of the Algerian desert.

The objective of the present study was to isolate and identify actinobacteria strains from rhizosphere of indigenous plants *Ononis angustisima* Lam. and *Astragalus gombo* growing in extreme environment (Sahara of Algeria) with the aim of testing their antimicrobial activity against pathogenic microorganisms and their biocontrol potential towards *Fusarium culmorum*, a pathogenic fungus of Wheat (*Triticum aestivum* L.).

MATERIALS AND METHODS

Sampling: The Rhizospheric soil of indigenous plants, *Ononis angustissima* Lam. and *Astragalus gombo* Coss. & Dur., was taken from Southern of Algeria (Biskra), which is located in the Saharan region (Figure 1). Samples were collected in sterile cylinders, closed tightly and stored in the refrigerator at 4°C until use. For each collected sample, 10 g of the soil was suspended in 90 ml of physiological water (NaCl 9 g/l), then incubated in an orbital shaker incubator at 50°C with shaking for 30 min at 150 rpm. The suspension was serially diluted up to10^{-6}. An aliquot of 0.1 ml of the appropriate dilutions was taken and spread evenly over the surface of yeast extract-malt extract agar medium (ISP2) (International Streptomycete Project) [27], supplemented with streptomycin (2.5 mg/ml) and amphotericin B (75 mg/ml) to inhibit bacterial and fungal contamination. Plates were incubated at 28°C and growth development was monitored through 14 days. The isolates were maintained on ISP2 medium slants at 4°C and as a glycerol suspension 20 % (v/v) at -20°C.

Phenotypic Characteristics of the 12 *Streptomyces* Strains: The morphology of the isolates was examined according to the methods recommended by Shirling and Gottlieb for the International Streptomycete Project (ISP) [27] and Bergey's manual of Systematic Bacteriology [28]. Visual observation using light microscopy and Gram-straining were performed for further identification. Cultural characteristics of pure isolates in various media (ISP2, ISP3, ISP4, ISP5, ISP6 and ISP7) were recorded after incubation for 7 to 14 days at 28°C. Catalase and oxidase activities were determined with 3 % (v/v) hydrogen peroxide solution and 1% (v/v) tetramethyl p-phenylenediamine dihydrochloride solution, respectively. The color of mycelium and soluble pigment were examined in ISP7 medium and determined by comparison with the color of chips in the Color Harmony Manual [29]. Growth at various pH values (4 to 11), the tolerance to NaCl (for 1 %, to 12 %) and the temperature range for growth (5°C to 50°C) were examined on ISP2.

***In vitro* Antagonistic Bioassay:** The following test microorganisms were used to evaluate the antagonistic bioassay of 12 actinobacteria: *Bacillus subtilis* ATCC 6633 (American Type Culture Collection, Manassas, VA, USA), *Escherichia coli* ATCC 25922, *Pseudomonas syringae* pv. tomato 1086, *Fusarium oxysporum* f.sp.*albedinis* and *Mucor ramannianus*. The actinobacteria were spot-inoculated onto ISP2 medium [27] and incubated at 28°C for 14 days. After this period, the antagonism between actinobacterias and the test microorganisms was evaluated using the streak assay [26]. All plates were incubated at 30°C and incubation time of 24–48 h for bacteria, 48–72 h for yeast and 7–10 days for fungi. All experiments were carried out in three replicates.

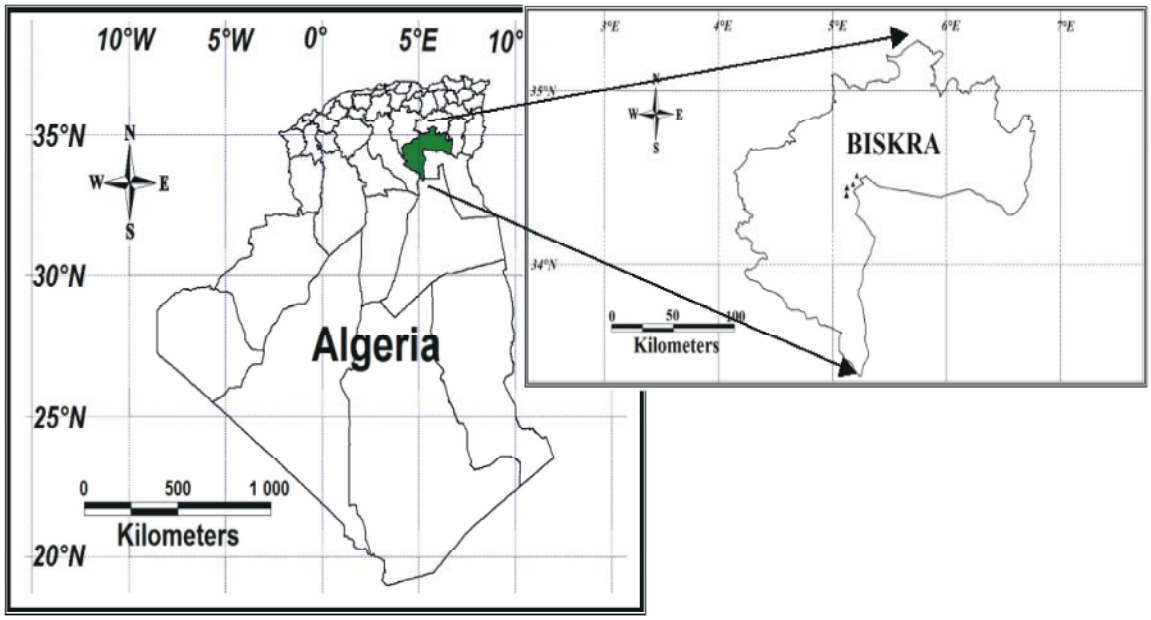

Fig. 1: A map showing the sites from which the rhizosphere soil samples of *Ononis angustissima* and *Astragalus gombo* were collected

Indole Acetic Acid (IAA) Production: The ability of six actinobacteria to produce IAA was measured based on the colorimetric method described by Khamna *et al.* [30] and Kaur et *al.* [31], with some modifications. Three 6 mm discs of growing actinobacterias from yeast malt agar were inoculated into100 ml YM broth containing 0.05 % L-tryptophan and incubated at 28°C on a rotary shaker at 160 rpm for 7 days. Cultures were harvested and centrifuged at 10,000×g for 10 min at 4°C. There action mixture, which included 2 ml of supernatant and 2 ml of Salkowski reagent, was incubated at 25°C for 30 min in the dark. Absorbance of the reaction mixture was measured at 530 nm and the IAA content (mg ml^{-1}) of the culture filtrate was quantified using a standard curve with known concentrations of pure IAA (Sigma).

Phosphate Solubility: Phosphate solubility was conducted qualitatively according to the method described by Franco-Correa *et al*. [32] and Piromyou *et al*. [33]. Twelve Actinobacteria were spot-inoculated onto minimal medium based on the Pikovskaya (PVK) medium described by Pikovskaya [34]. This medium contained (per liter): glucose, 10 g; $Ca_3(PO_4)_2$, 5 g; $(NH_4)_2SO_4$, 0.5 g; NaCl, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; KCl, 0.2 g; yeast extract, 0.5 g; $MnSO_4 \cdot H_2O$, 0.002 g; and $FeSO_4 \cdot 7H_2O$, 0.002 g, supplemented with agar 10 g. The dishes were incubated at 28°C for 7 days. A positive reaction was indicated by clear zones around the colonies.

Protease Production: It was done as per the protocols of Bhattacharya et *al*. [35]. Actinobacteria were streaked on casein agar medium and incubated at 28°C for 7 days. At the end of the incubation, the plates were observed for halo zone around the colonies, which indicates the presence of protease.

Chitinase Production: Chitinase activity was examined using the modified method described by Gupta *et al*. [36] and Kawase et *al*. [37]. The chitinase enzyme activity of the 12 selected isolates was tested in nutrient agar medium containing 1% colloidal chitin. Chitinase production was assessed by visual examination of cleared zones developed around colonies incubated at 28°C for 7 days.

***In vitro* Biocontrol Essay:** The cultivar of wheat (*Triticum aestivum* L.), namely 'HD 1220', highly susceptible to Fusarium head blight were used in experiments. The seeds were surface sterilized by immersion in 70 % ethanol for 1 min, followed by continuous agitation in a 1 % sodium hypochlorite for 5 min and rinsed with sterile distilled water. Seeds were allowed to imbibe water overnight at room temperature. Pre-germinated seeds were grown in Petri dishes. The Petri dishes experiments were performed in environmental controlled conditions using plant growth chamber. Seeds were germinated in 150 mm diameter plastic Petri

dishes each containing two layers of 125-mm diameter Whatman N°1 filter paper (20 seeds per Petri dish) moistened with 8 ml Murashige and Skoog (MS) solution [38].in three replicates. Six actives fresh suspensions of *Streptomyces* spp. approximately 1×10^6 CFU/ml in 1 ml of ISP2 broth with 0.01 % Tween-20 and 1 ml of *F. culmorum* approximately 1×10^5 CFU/ml were added to the seeds immediately before planting. Plants without *Streptomyces* spp. strains and *F. culmorum* served as negative control and plants with *F. culmorum* and Tebuconazole (60g/l) served as positive control. Plants were maintained in a growth room conditions with temperature 24°C, 16 h light / 8 h dark photoperiod and relative humidity of 80 % and fertilized weekly with a Murashige and Skoog (MS) solution [38]. The germination index and growth parameters: shoot height, shoot weight (fresh), root weight (fresh) and root length were determined after 4 week. The experiment was conducted with three replicates per treatment.

Statistical Analyses: Data from the antimicrobial activity assay and biocontrol test of the six *Streptomyces* spp. strains were analyzed by SAS software 9. Means of treatments for each experiment were compared by using Duncan Multiple Range Test ($P \leq 0.05$).

RESULTS AND DISCUSSION

The actinobacteria strains were isolated from the rhizosphere of *Ononis angustissima* and *Astragalus gombo* grown in the Biskra, Algeria. The ISP2 agar medium added with supplemented with streptomycin and amphotericin B was selected for actinobacteria isolation from rhizospheric soil samples. It was found that 50°C for 30 min was the best heat regimen for recovering sporoformer actinobacterias after heat treatments of the rhizospheric soil. A number of actinobacteria colonies with different morphological and cultural characteristics were picked from 2 week-old isolation plates and transferred to ISP2 medium for purification and morphology observation.

A total of 120 actinobacteria isolates were obtained from the *Ononis angustissima* and *Astragalus gombo* rhizosphere soil obtained from Southern of Algeria (Biskra), only twelve isolates were selected and confirmed as *Streptomyces* spp. based on morphological and cultural characteristics.

The identification of the newly actinobacteria was based on phenotypic methods. Morphological, biochemical and physiological characteristics, according to the methods described in Bergey's Manual of Systematic Bacteriology [28] and International Streptomycete Project (ISP) [27], showed that the isolated strains appear aerobic, Gram-positive, non-motile, catalase and oxidase positive, that form extensively branched aerial and substrate hyphae. The aerial mycelium is grey to white in color and the substrate mycelium appears light yellow to brown. Optimum growth occurs at 28°C. The pH range for growth was pH 4–11, with optimum growth at pH 7. Tolerates to NaCl concentrations up to 10 %. Soluble pigments are generated on ISP 2, ISP 3, ISP 5 and ISP 7. Melanin production was detected by ON03, BI16, BI22, BI25, ON10 and ON15 isolates but was not detected by ON20, BI26, BI28, OR6, ON02 and BI15 strains. According to the morphology of the spore chains observed under light microscopy, were as spiral shaped. All the data obtained with regard to the physiological and biochemical properties of the isolates, therefore, strongly confirmed that the strains ON03, BI16, BI22, BI25, ON10, ON15, ON20, BI26, BI28, OR6, ON02 and BI15 belonged to the *Streptomyces* genus.

Screening of Phosphate Solubilizing Actinobacteria: Qualitative estimation of P solubilization by actinobacterias strains grown on Pikovskaya medium showed the development of a clear solubilization zone around the colony. In this study, 12 isolates were evaluated and 5 (40,67%) isolates showed P-solubilizing activity in 7 days (Table 1). The four isolates BI15, BI26, BI28, ON02 and ON03 were able to solubilise phosphate which was confirmed by appearance of largest halos around their colonies (translucent areas) in PVK agar medium.

Phosphorus is considered as growth-limiting macronutrient. Phosphate solubilizing microorganisms have been employed in agriculture and horticulture and have been considered very important due to their potential of ecological amelioration [39]. The actinobacteria strains tested seems to have the ability of solubilizing P sources in soil. Among the several potential mechanisms for phosphate solubilization those involving the production of chelating compounds, like organic acids or by means of a modification of pH of the medium by the secretion of organic acids or protons are the more often described in the literature [34, 40, 32]. The potential for phosphate solubilization of the target actinobacteria supports future research for quantitative analysis.

Production of Plant Growth Promoting Hormone Indole Acetic Acid: The ability of the bacterial strains to produce IAA was detected by the development of pink color in ISP2 culture medium after the addition of salkowski reagen

Table 1: Evaluation of qualitative hydrolytic enzyme production (chitinase and protease) and phosphate solubilization by *Streptomyces* spp. isolated from the rhizosphere of *Ononis angustissima* and *Astragalus gombo*

Rhizosphere	Strains	Chitinase[a]	Protease[a]	Phosphate solubilization [b]
Astragalus gombo	BI15	+	+	++
	BI26	+	+	+
	BI28	+	-	++
	BI22	-	-	-
	BI25	-	-	-
	BI16	-	-	-
Ononis angustisima	ON03	+	+	+++
	ON02	+	+	+++
	ON10	-	-	-
	ON20	-	-	-
	ON15	-	-	-
	OR6	+	-	-
Percentage (%)		50	33,33	41,67

[a]+: presence of halo zone around the actinobacteria colonies, -: absence of halo zone around the actinobacteria colonies.

[b]+++: solubilisation halo=10mm; ++: 5mm< halo <10mm; +: halo <5mm; -: no solubilisation halo

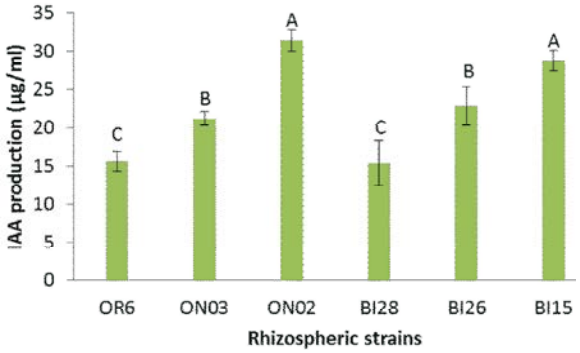

Fig. 2: Indole acetic acid (IAA) production by the six most potential antagonistic *Streptomyces* spp. The results are mean values of three data sets, (Duncan's test, $p < 0.05$), different lowercase letters on top of the histograms indicate significant differences

to the culture. Among 12 *Streptomyces* spp. strains, 6 were able to produce high levels of IAA. Interestingly, *Streptomyces* spp. strains ON02 and BI15 produced highest amount of IAA as compared to the four other strains (Figure 2).

IAA affects plant cell division, extension and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light and gravity; affects photosynthesis, pigment formation, biosynthesis of various metabolites and resistance to stressful conditions [41, 42]. IAA production is another attribute that have very vital role in the plant growth promotion potential of these strains.

Production of Fungal Cell Wall-Degrading Enzymes: Several properties such as cell wall degrading enzyme production have been heavily reported as important mechanisms of antifungal action for various *Streptomyces* species [43, 44, 45, 46]. In this study, the production of fungal cell wall-degrading enzymes was examined because this is an important mechanism of fungal inhibition for PGPR agent. The chitinase production was shown for half of the strains (Table 1). Six (50%) rhizosphere strains showed fungal cell wall-degrading enzyme chitinase activity. Proteolytic activity was detected for four of 12 (33,33%). In general, the higher chitinase activity was correlated with higher fungal inhibition. For this reason, chitinolytic *Streptomyces* strains are a likely choice as potential biological control agents.

It has been reported that antifungal mechanism of antagonists has been attributed to the action of hydrolytic enzymes such as chitinase, [47] and protease [48].

Antagonisms: Actinobacteria strains exhibiting the ability to produce both clear zones of inhibition and metabolites against the tested pathogenic bacteria and fungi were considered antagonistic. Total 120 actinobacteria isolates

Table 2: Screening of *Streptomyces* spp. strains for their antagonistic as measured by diameter of the inhibition zone of the indicator strain (mm)

	Diameter of inhibition zone (mm)*					
	Indicator strains					
Streptomyces sp. strains	*F. oxysporum* f.sp.*albedinis*	*F. culmorum*	*M. ramannianus*	*B. subtilis*	*P. syringae*	*E. coli*
BI15	$12,7\pm2,5^{d}$	$18,7\pm1,1^{b}$	$12,3\pm2,5^{cb}$	$10,7\pm1,1^{f}$	$14,3\pm0,6^{dc}$	$16,3\pm1,5^{e}$
BI26	$23,3\pm3,1^{c}$	$15,7\pm2,1^{b}$	$13,7\pm1,5^{b}$	$18,3\pm1,5^{e}$	$11,0\pm1,7^{f}$	$15,7\pm2,1^{fe}$
BI28	$27,3\pm2,5^{b}$	$18,0\pm2^{b}$	$22,0\pm2,0^{a}$	$23,7\pm3,1^{dc}$	$19,0\pm1,0^{b}$	$28,7\pm1,1^{b}$
BI22	$5,0\pm1g^{f}$	$4,7\pm1,1^{dc}$	$8,7\pm1,5^{d}$	$30,7\pm1,1^{b}$	$19,0\pm1,0^{b}$	$32,7\pm2,1^{a}$
BI25	$9,0\pm1^{ed}$	$3,3\pm1,5^{d}$	$8,7\pm1,5^{d}$	$16,3\pm1,5^{e}$	$18,3\pm1,5^{b}$	$15,3\pm1,5^{fe}$
BI16	$9,3\pm1,1^{d}$	$7,0\pm1^{c}$	$4,3\pm1,1^{e}$	$25,7\pm1,5^{c}$	$24,0\pm1,0^{a}$	$29,7\pm1,5^{ba}$
ON03	$10,3\pm1,5^{d}$	$22,7\pm2,5^{a}$	$12,0\pm2,6^{cbd}$	$22,7\pm3,1^{dc}$	$13,7\pm1,1^{dce}$	$22,3\pm2,5^{c}$
ON02	$30,7\pm1,1^{a}$	$25,3\pm2,5^{a}$	$23,7\pm1,5^{a}$	$32,3\pm2,5^{ba}$	$25,3\pm1,5^{a}$	$19,7\pm1,5^{dc}$
ON10	$7,7\pm0,6^{edf}$	$4,3\pm0,6^{dc}$	$9,3\pm0,6^{cd}$	$35,3\pm0,6^{a}$	$7,7\pm1,5^{g}$	$22,0\pm2,0^{c}$
ON20	$8,0\pm1^{ed}$	$5,0\pm1^{dc}$	$9,7\pm1,5^{cd}$	$9,0\pm1,0^{f}$	$12,3\pm2,5^{dfe}$	$10,0\pm1,0^{g}$
ON15	$4,7\pm0,6^{g}$	$2,7\pm0,6^{d}$	$4,3\pm0,6^{e}$	$10,3\pm0,6^{f}$	$11,7\pm1,1^{fe}$	$12,7\pm2,1^{fg}$
OR6	$6,3\pm1,5^{egf}$	$17,7\pm2,5^{b}$	$15,0\pm3^{b}$	$22,3\pm2,5^{d}$	$15,0\pm1,0^{c}$	$17,7\pm2,5^{de}$

*: Values in the table are means of three independent experiments and error bars indicates standard deviation of the mean. Letters show significant deference using Duncan's test ($p < 0.05$)

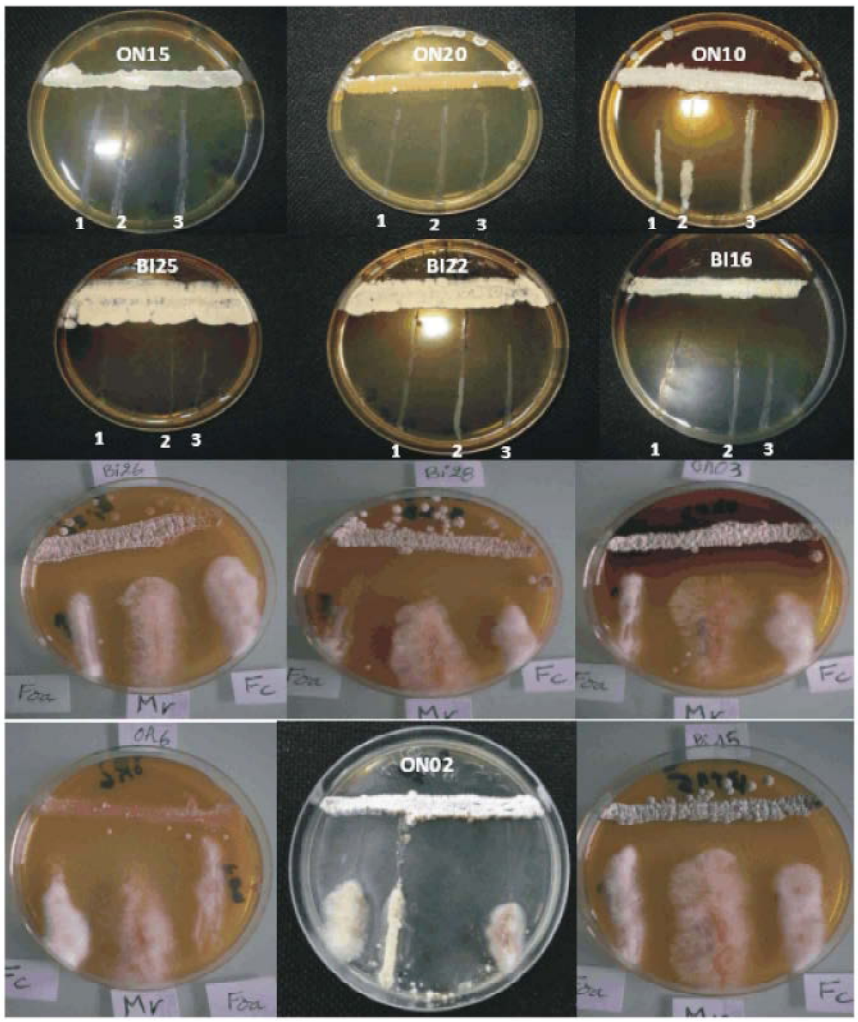

Fig. 3: Antagonistic activity *in vitro* of *Streptomyces* spp. isolates against *P. syringae* (1), *B. subtilis* (2) and *E. coli* (3), *F. oxysporum f.sp.albedinis* (Foa), *M. ramannianus* (Mr) and *F. culmorum* (Fc)

were isolated from rhizosphere of *Ononis angustissima* and *Astragalus gombo* growing in Biskra, Algeria and only twelve (ON03, BI16, BI22, BI25, ON10, ON15, ON20, BI26, BI28, OR6, ON02 and BI15) exhibited antimicrobial activity. These twelve strains showed strong antibacterial activity against Gram-positive bacteria (*B. subtilis*), Gram-negative bacteria (*P. syringae* and *E.coli*) and fungal pathogen (*F. culmorum*, *F. oxysporum* and *M. ramannianus*) (Figure 3). Among the all bacterial pathogens, maximum growth inhibition (35,3±0,6 mm) was recorded with *B. subtilis* followed by *E.coli* (32,7±2,1 mm) and *P. syringae* (25,3±1 mm) (Table 2).

Furthermore, the strain ON02 exhibited maximum antifungal activity (30,7±1,1 mm inhibition zone) with *F. oxysporum* followed by ON02 and ON03 (25,3±2,5 mm and 22,7±2,5 mm respectively) against *F. culmorum* and ON02 and BI28 (23,7±1,5 mm and 22,0±2,0 mm respectively) with *M. ramannianus* (Table 2). The broad-spectrum activity exhibited by some of the isolates is possibly due to the production of diverse antimicrobial compounds. Like in earlier studies, the *Streptomyces* strains we isolated possessed more antifungal than antibacterial properties [49, 50]. The antagonism of *Streptomyces* observed in this study is in accordance with previous reports [51, 30]. According to [51], *Streptomyces* sp. TK-VL_333 showed antagonistic activity toward a variety of bacteria, yeast and filamentous fungi. Verma *et al*. [52], observed that approximately 60 % of the isolated actinobacterias showed wide-spectrum antimicrobial activity against bacteria and fungi. These results confirm that the actinobacterias are able to produce a wide variety of antibiotics and other compounds with antibacterial and antifungal activity. These results indicate that the rhizosphere of *Ononis angustissima* and *Astragalus gombo* is a useful potential source for isolation of actinobacterias producers of biological active products.

Biocontrol Agents: Microbial antagonists are widely used for the biocontrol of fungal plant diseases. Many species of actinobacteria, particularly those belonging to the genus *Streptomyces*, are well known as antifungal biocontrol agents that inhibit several plant pathogenic fungi. The results in this study indicate that seed germination was strongly inhibited when treated with pathogenic fungi (Figure 4G). The findings of the current study also indicated that the wheat cultivar 'Hadba HD 1220' seedlings coating *F. culmorum* treated with inoculum of ON03, BI26, BI28, OR6, ON02 and BI15 strains with a double character plants growth promoting rhizobacteria (PGPR) and biocontrol agent, increase significantly the percentage of germination as compared to no inoculated or inoculated with pathogen alone or treated with tebuconazole (60g/l) consecutively (Figure 4).

Increased index of germination was observed from the wheat seeds indicating the potential of *Streptomyces* spp. strains to inhibit pathogenicity and by this means increase germination. A collective effect of many factors, such as production of antifungal substances [8, 9] and phytohormone [8, 9, 41] by *Steptomyces* spp. strains, might be involved in increasing seed germination and controlling plant pathogenicity [45, 46]. Few or most of these factors attribute an advantage to treated plants.

The potential use of *Streptomyces* spp. as a biocontrol agents has been reviewed previously [45, 46], where inoculation with these microorganisms generally promoted growth of plants. Therefore, plant height was measured after 30 days of growth and a significant increase in root length and shoot height was observed in all treatments compared with their respective controls, showing that the all *Streptomyces* spp. strains were able to promote growth of plants (Figure 6).

Almost all rhizospheric *Streptomyces* strains enhanced the agronomic performance of wheat cultivar 'Hadba HD 1220' by influencing its growth parameters. The six strains *Streptomyces* spp. showed significant increases in root length (Figure 6a). The longest root length (64 mm) was attained by the strain NO02 followed by strain BI15 (61 mm) but the shortest one was observed of the plant inoculated with *F. culmorum* (5,7 mm). Significant increases of root fresh weight were achieved by the six strains of which the tow strains BI15 and BI28 (36 mg and 32 mg respectively) followed by OR6 and BI26 (25,33 mg, 25 mg and 20,67 mg gave the highest record (Figure 6b).

More or less the same observation was recorded for shoot growth criteria, where ON03, BI26, BI28, OR6, ON02 and BI15 *Streptomyces* strains significantly improved shoot length and fresh weight of wheat by the range of 88,10– 62,70 mm and 42,53–24,30 mg respectively (Figure 6c, d). The maximum shoot promoting effects were performed with the strains ON02 (88.10 mm, 42,53 mg for height and fresh weight, respectively) and BI15 (82,73 mm and 40,07 mg for height and fresh weight, respectively). In the whole, the strains ON03, BI26, BI28, OR6, ON02 and BI15 significantly increased all measured parameters.

The germinative energy can play an important role in the achievement of quick and uniform seedling emergence and the reduction of damping-off incidences, thus improving the yield. Khaleeq and Khan [53], reported that

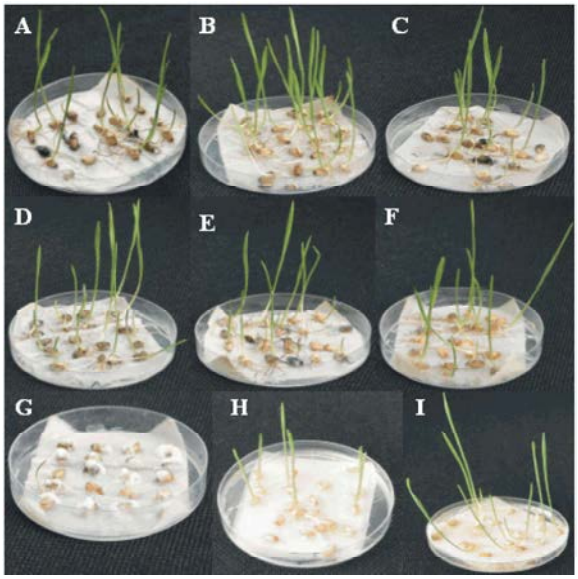

Fig. 4: Biological control of Fusarium head blight (FHB) in Wheat seeds treated with *Streptomyces* spp. strains ON03 (A), ON02 (B), OR6 (C), BI15 (D), BI26 (E), BI28 (F) and effect of *F. culmorum* (G) and tebuconazole (H). I: no treated plants

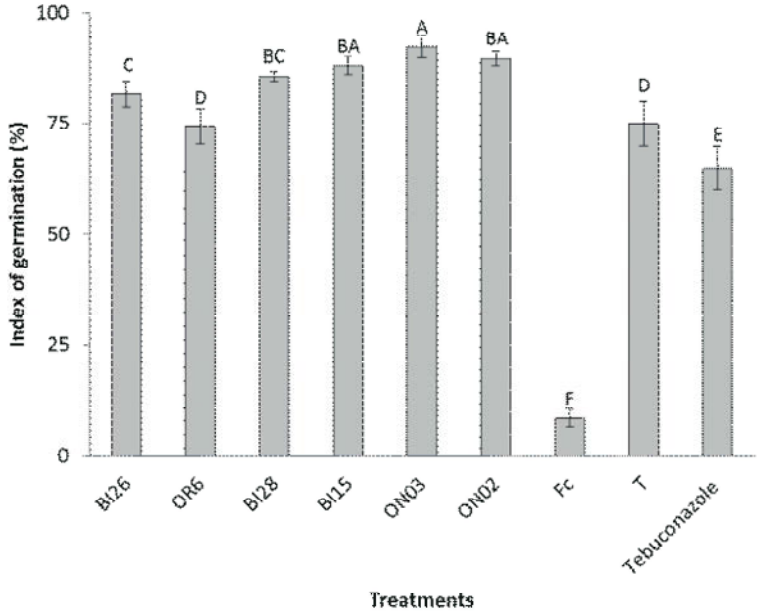

Fig. 5: Effect of *Streptomyces* spp. strains (21, 1B10, 2A26 and 2C34) on the germination index of wheat (*Triticum aestivum* L.) inoculated with pathogenic fungus (*F. culmorum*). Duncan's test ($p > 0.05$) showed that the histograms using different letters were significantly different. Values are means of three independent experiments and error bars indicates standard deviation. T: no inoculated plants, Fc: *F. culmorum*

the use of fungicides is effective in enhancing germination, emergence and growth as well as in reducing damping-off. In addition, accelerated germination is reported to help improve stress resistance and enhance overall plant growth and productivity [54, 55, 56]. The results obtained for the IAA, chitinase and antifungal compounds production, by the four *Streptomyces* strains showing a positive reaction. It should be noted that one of the possible antifungal mechanisms of the strains ON03, BI26, BI28, OR6, ON02 and BI15 may be associated with the production of antifungal agent, extracellular chitinase enzymes and IAA. *Streptomyces* species have

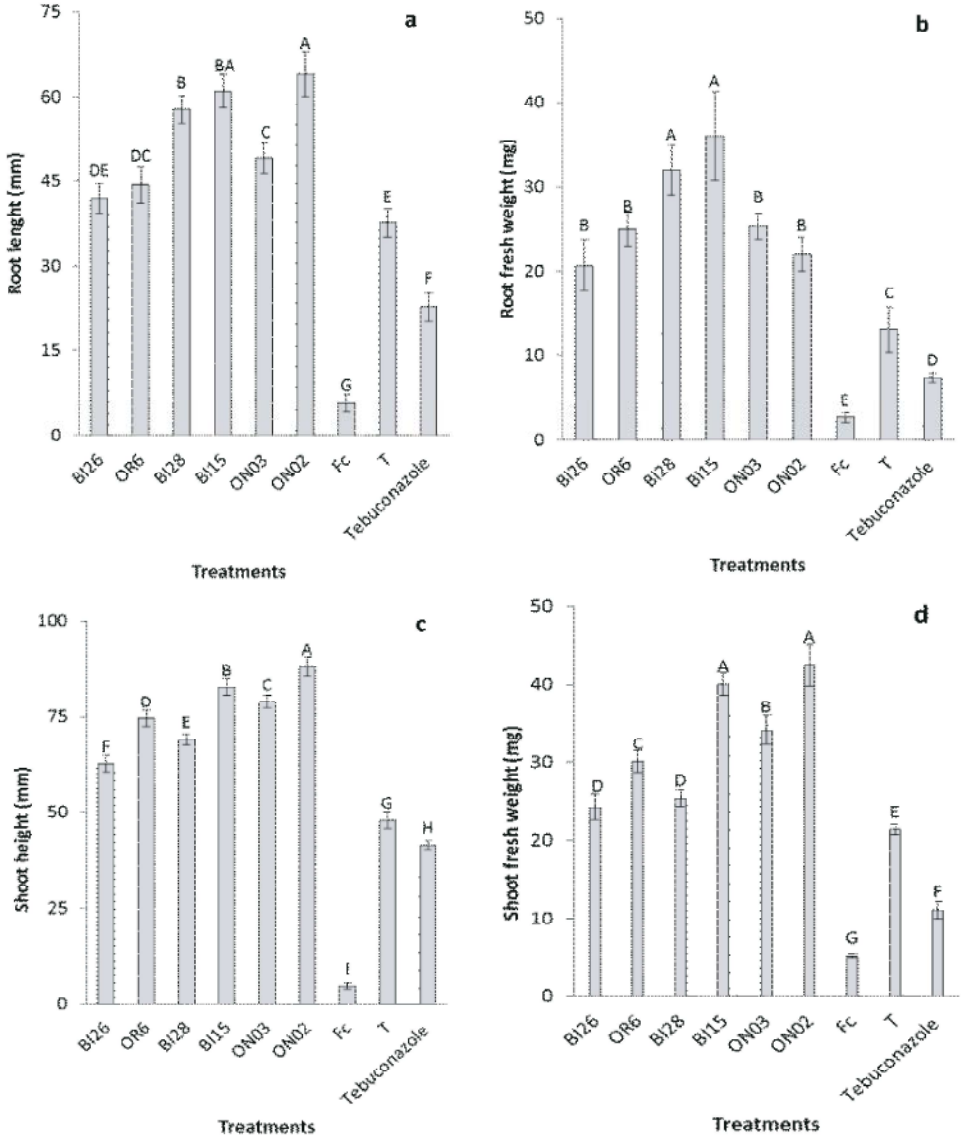

Fig. 6: Effect of *Strptomyces* sp. strains on the, root length (a), root weight (b) shoot height (c) shoot weight (d) of wheat (*Triticum aestivum* L.) inoculated with pathogenic fungus (*Fusarium culmorum*). Duncan's test ($p < 0.05$) showed that the histograms using different letters were significantly different. Values are means of three independent experiments and error bars indicates standard deviation. Control negative (no inoculated plants), Fc: *F. culmorum*, T: Plants without *Streptomyces* spp. strains and *F. culmorum*.

been reported as biocontrol agents effective against numerous plant pathogens [57, 58, 59, 60, 8, 61]. Thus, these *Streptomyces* spp. strains can also play a role in plant development, considering that growth promotion effects may be related to IAA production [30, 62], as reported in other studies, this protective effect might be increased by the ability of the selected actinobacteria strains to excrete chitinases [63], nitrogen fixation [32], siderophores production [64, 65] or other antifungal substances [66, 67]. Singh and Chhatpar [22], also attributed the activity of *Streptomyces* sp. A6 against fungal plant pathogens to production of mycolytic enzymes and an unknown antifungal metabolite.

CONCLUSION

The isolates *Streptomyces* spp. ON03, BI26, BI28, OR6, ON02 and BI15 isolated from a rhizospheric soil of *Ononis angustissima* and *Astragalus gombo* growing in Algeria (Biskra) showed a broad range of antimicrobial

activity. The isolates were found to be a *Streptomyces* spp. based on the phenotypic characteristics.

Biocontrol potential of the isolates was determined by its antimicrobial activity, IAA production, chitinase activity and phosphate solubility. Results of the present study indicated that these isolates clearly have a potential as biological control agents against *F. culmorum*, which have signi?cant suppressive effect on root infecting fungi, and plant growth-promoting effect of wheat under controlled conditions. This confirms the properties of isolated actinobacteria to promote plants growth. Further studies are necessary, to evaluate the effect of their biocontrol potential in greenhouse and field conditions, and characterize the enzymes and secondary metabolites produced by these *Streptomyces*.

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