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REGULAR ARTICLE

Isolation and characterization of rhizospheric *Streptomyces* spp. for the biocontrol of Fusarium wilt (bayoud) disease of date palm (*Phoenix dactylifera* L.)

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Abstract

Fusarium wilt of date palm (*Phoenix dactylifera* L.) caused by *Fusarium oxysporum* f.sp. Albedinis is one of the most destructive diseases in North Africa spicily in Algeria and Morocco. The objectives of this work were the isolation, identification and biocontrol efficacies testing of rhizospheric actinobacteria of medicinal plant *Astragalus gombo* against fusarium wilt disease in date palm. Five rhizospheric *Streptomyces* spp. Isolates bi02, bi21, bi24, bi26 and bi28 showed the largest zone of inhibition demonstrating a potent effect against *F. oxysporum* f.sp. Albedinis and a broad spectrum of antimicrobial activities against the test microorganisms. All these selected isolates produced indol acetic acid (IAA) at different levels, exhibited higher activity of phosphate solubilisation and siderophore production as well as chitinase and protease activities. The inoculation of infected date palm with the five *Streptomyces* spp. Increased all parameters of growth measured in controlled conditions. The study revealed that the five *Streptomyces* spp. could be a potential biocontrol agent for controlling palm and also promoting growth of plants.

Key words: Antimicrobial, phosphate solubilization, siderophore, promoting growth of plants

Introduction

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous plant which belongs to the family Arecaceae. It has a long history of cultivation and utilization in North Africa and Middle East. This plant has immense socio-economic (an essential resource for food security), environmental and ecological values (key reservoir of biodiversity), particularly in the arid and semi-arid regions of the world (Kriaa et al., 2012; Idder et al., 2015). In Algeria, around 18 million date palms are cultivated and producing an annual yield of 500,000 mt of dates (Bouguedoura et al., 2015).

Date palms are vulnerable to number of disease problems. The Fusarium wilt of date palm, also known as bayoud, caused by a soil fungus *Fusarium oxysporum* f.sp. *albedinis*

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(Killian and Maire) Gordon (Ait Kettout and Rahmania, 2010), is the most destructive disease in North Africa, having no effective control strategy (Brac de la Perri et al., 1995; El Hassni et al., 2007).

Since 1898, diseases like this destroyed millions palm trees in south-western Algeria and still a major threat to date palm trees (Boufis et al., 2014). Chemical pesticide usage is generally accepted as effective strategy, but there is lack of effective chemical product for Fusarium wilt in date palm. Apart from this there are side effects associated with chemical usage (Karlidag et al., 2007; Sowndhararajan et al., 2012; Sathiyabama and Charles, 2015). And also there are acquired resistance in pathogenic bacteria and fungi to commonly used strategies (Kumar et al., 2011). Hence, it is need of hour to search and develop non-hazardous biologically compatible alternatives. Application of antagonistic microorganisms to combat plant pathogens is seeming to be a better alternative and sustainable management strategy for plant protection. Inhibition or killing of harmful pests by biocontrol agents is biologically safe, target specific and non-polluting (Karthik et al., 2015).

Application of plant growth promoting Rhizobacteria (PGPR) on datepalm can be used as an effective biocontrol strategy against this conditions. The PGPR application will enhance the fertility of soil and plant growth and can ultimately inhibit the pathogens (Gupta et al., 2015; Mathivanan et al., 2017). The nitrogen from the atmosphere can be fixed by the PGPR which in turn increase the plants ability to absorb nitrogen and increase its growth (Groblelak et al., 2015). The mechanisms for plant disease control include competition for space and nutrients among antagonist and pathogen, production of antimicrobial compounds (Chauhan et al., 2015) and induction of systemic resistance in host (Lee et al., 2015). PGPR are also capable of the producing secondary metabolites having antibiotic, antifungal, insecticidal and immunosuppressants properties (Groblelak et al., 2015).

Streptomyces is a genus of Gram-positive bacteria, with a filamentous form similar to fungi. There are differences in morphology of *Streptomyces* which involves the formation of a layer of hyphae that can differentiate into a chain of spores. This bacterium can produce bioactive secondary metabolites such as antifungals, antivirals, antitumoral, anti-hypertensives, and mainly antibiotics and immunosuppressive (De Lima Procopio et al., 2012). Actinomycetous bacteria have been recognized as sources for several secondary metabolites, antibiotics, and lytic enzymes of medical and industrial value. Nearly 70 % of the natural antibiotics used in clinical practices are being produced by actinobacteria (Subramani and Aalbersberg, 2015) of which 75–80% have been derived from *Streptomyces* alone (Khieu et al., 2015).

Considering the inherent beneficial characteristics of rhizospheric actinobacteria, the objective of this study was to select of *Streptomyces* spp. isolated from rhizosphere of indigenous legume *Astragalus gombo* in Algeria with potential for biocontrol of Bayoud disease caused by *Fusarium oxysporum* f. sp. *albedinis* and for promoting the growth of date palm (*Phoenix dactylifera* L.) under controlled conditions.

Material and methods

Sampling and identification of actinobacteria

Actinobacteria was isolated from the rhizospheric soil of indigenous legume *Astragalus gombo* Coss. & Dur. growing in the desert region Biskra of Southern Algeria (Figure 1). This medicinal plant grows in sandy arid and desert pastures of Algeria and rarely exploited. Samples were collected in sterile cylinders, closed tightly and stored in the refrigerator at 4°C until use as explained by Ghadbane et al., (2015). 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 were taken and spread evenly over the surface of yeast extract-malt extract agar medium (ISP2) (International Streptomycete Project) (Shirling and Gottlieb, 1966), supplemented with streptomycin (2.5 mg/ml) and amphotericin B (75 mg/ml) to inhibit bacterial and fungal contamination, respectively. 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.

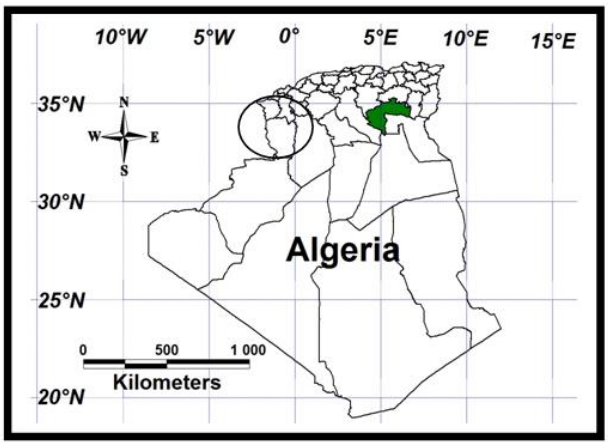

Fig. 1. A map showing the sites from which the rhizosphere soil samples of *Astragalus gombo* were collected.

Phenotypic characteristics of the *Streptomyces* strains

The morphology of the isolates was examined according to the methods recommended by Shirling and Gottlieb for the International Streptomycete Project (ISP) (Shirling and Gottlieb, 1966) and Bergey's manual of Systematic Bacteriology (Williams et al., 1989). Visual observation using light microscopy and Gram-straining were performed for further identification. Cultural characteristics of pure isolates were recorded after incubation for 7 to 14 days at 28 °C in various media: ISP1 (tryptone-yeast extract), ISP2 (yeast extract-malt extract agar), ISP4 (inorganic salts-starch agar), ISP5 (glycerol-asparagine agar), ISP6 (peptone-yeast extract iron agar) and ISP7 (tyrosine agar). 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 (Jacobson et al., 1958). Carbohydrate utilization was determined by growth on carbon utilization medium (ISP9) (Shirling and Gottlieb, 1966) supplemented with 1 % carbon sources at 28 °C. Growth at various pH values (4 to 11), the tolerance to NaCl (for 1 %, to 13 %) and the temperature range for growth (10 °C to 50 °C) were examined on ISP2.

***In vitro* antagonistic bioassay**

Five actinobacteria were used to antagonistic bioassay against following pathogens: *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, F. culmorum* and *Mucor ramannianus*. The antimicrobial activity of actinobacteria isolates was performed by using the cross streak modified method (Kumar et al., 2014; ghadbane et al., 2015). ISP2 plates were prepared and inoculated with isolates by a single streak in the Petri plate and incubated at 30°C for 7 days. The plates were then inoculated with the test organisms by a single streak at 90° angles to actinobacteria strains and incubated at 37°C overnight for bacteria, and at 24°C of 7–10 days for filamentous fungi. Antagonism was observed by the inhibition of the test organism. All experiments were carried out in three replicates.

Protease production

It was done as per the protocols of Bhattacharya et al. (2009). 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. (1995) and Kawase et al. (2004). The chitinase enzyme activity of the 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.

Siderophore production

Siderophore production was tested qualitatively using the modified method described by Schwyn and Neilands (1987) and Simonettia et al. (2015) in which chrome azurol sulphonate (CAS) medium was cast upon Luria Bertani (LB) plates containing cultivated actinobacteria. Colonies with yellow–orange zones were considered as siderophore-producing isolates.

Determination of potential plant growth promoting traits of selected *Streptomyces*

The ability of actinobacteria to produce IAA was measured based on the colorimetric

method described by Khamna et al. (2009) and Kaur et al. (2013), 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 was conducted qualitatively according to the method described by Franco-Correa et al. (2010) and Piromyou et al. (2011). Actinobacteria were spot-inoculated onto minimal medium based on the Pikovskaya (PVK) medium described by Pikovskaya (1948). 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.

Biological control assay

The biocontrol efficacy and growth promoting effects of five *Streptomyces* spp. isolates were studied in growth room conditions with temperature 24 °C, 16 h light / 8 h dark photoperiod and relative humidity of 80 %. Pre-germinated seeds were grown in pots containing sand–peat-soil mixture (1:1:1 v/v/v). A fresh suspension of *Streptomyces* spp. (Strains BI02, BI21, BI24, BI26 and BI28) approximately 1×10^6 CFU/ml in 1 ml of ISP2 broth with 0.01 % Tween-20 and 1 ml of *F. oxysporum* f.sp. *albedinis* approximately 1×10^5 CFU/ml were added to the planting mixture immediately before planting. Plants without *Streptomyces* spp. strains and *F. oxysporum* f.sp. *albedinis* served as control. Seeds were placed on the surface of the planting mix approximately 2 cm from to the pot (five seedlings per pot) and covered with a 0.5 cm layer of the sterilized sand and vermiculite mixture. Plants were fertilized weekly with a Murashige and Skoog (MS) solution Murashige and Skoog (1962), and watered as needed. The germination index and growth parameters, shoot height, shoot fresh weight, root fresh weight and root length were determined after six months. The experiment was conducted with three replicates per treatment.

Statistical analyses

All results were analyzed statistically with SAS software 9. Statistical differences between means were determined by one-way ANOVA with Duncan's multiple range with the level of significance established at $P < 0.05$.

Results and discussion

Identification phenotype of actinobacteria

The actinobacteria strains were isolated from the rhizosphere of *Astragalus gombo* grown in Biskra, Algeria. The ISP2 agar medium added with supplemented with streptomycin and amphotericin B was selected for actinobacteria isolation from rhizospheric soil samples.

A total of 24 suspected actinobacteria isolates were obtained from the *Astragalus gombo* rhizospheric soil from Southern of Algeria (Biskra), and 5 isolates (BI02, BI21, BI24, BI26 and BI28) were selected (Fig. 2) and confirmed as *Streptomyces* spp. based on morphological and cultural characteristics (Table 1).

Phenotypic methods were mainly employed for the identification of the new actinobacteria and based on the methods described in Bergey's Manual of Systematic Bacteriology (Williams et al., 1989) and International Streptomycete Project (ISP) (Jacobson et al., 1958) showed that the isolated strains appear aerobic, Gram-positive filamentous bacteria forming long spore chains in the aerial mycelia, 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 or black. Optimum growth occurs at 28 °C. The pH range for growth was pH 6–11, with optimum growth at pH 7.

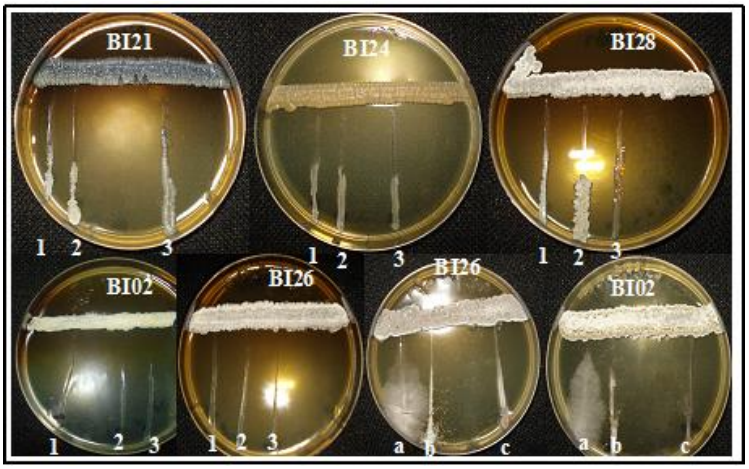

Fig. 2. Inhibitory activity of *Streptomyces* spp. BI21, BI24, BI28, BI02 and BI26 strains against (1) *Bacillus subtilis,* (2) *Escherichia coli* and (3) *Pseudomonas syringae,* and *Streptomyces* spp. BI02, BI21 against (a) *Fusarium oxysporum* f.sp. *albedinis* (b) *F. culmorum* and (c) *Mucor ramannianus*.

Table 1. Morphological and physiological characteristics of the five selective *Streptomyces* spp. Isolates.

Characteristic	*Streptomyces* spp.				
	BI02	BI21	BI24	BI26	BI28
Spore Chain morphology	Rectiflexibiles	Rectiflexibiles	Rectiflexibiles	Spiral	Spiral
Aerial hyphae	White	White	Grey	Grey	White
Melanin production	-	-	+	+	+
Maximum NaCl tolerance (%, w/v)	13	13	10	10	10
Colour of spore mass/substrate mycelium on:					
ISP1	White/Brown	White/Brown	Grey/Brown	White/Brown	White/brown
ISP2	White/yellow	White/yellow	White/Brown	White/Brown	Grey/brown
ISP4	Grey/Brown	Grey/Brown	White/Brown	White/yellow	Grey/brown
ISP5	Grey/Brown	White/yellow	White/Brown	White/yellow	White/black
ISP6	White/yellow	White/yellow	White/Brown	White/black	Grey/brown
ISP7	White/yellow	Grey/yellow	White/black	White/black	Grey/brown
Colour of soluble pigment on:					
ISP2	Brown	Brown	Yellow	Brown	Brown
ISP3	Yellow	Yellow	Brown	Brown	Brown
ISP4	Yellow	brown	Yellow	Yellow	Brown
ISP5	Yellow	Yellow	Brown	Yellow	Brown
ISP6	Yellow	Yellow	Brown	Black	Black
ISP7	Yellow	Yellow	Black	Black	Black
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	+
Temperatur °C :					
10	-	-	-	-	-
20	-	+	-	-	+
25	+	+	+	+	+
30	+	+	+	+	+
35	+	+	+	+	-
45	+	-	+	+	-
pH					
5	-	-	-	-	-
6	+	+	+	+	+
7	+	+	+	+	+
11	+	+	+	+	+

Tolerates to NaCl concentrations up to 10 for BI24, BI26 and BI28, and up to 13 % for BI02 and BI21. Soluble pigments are generated on ISP2, ISP3, ISP5 and ISP7. Melanin production was detected by BI24, BI26 and BI28 isolates but was not detected by BI02 and BI21 isolates (Table 1). According to the morphology of the spore chains observed under light microscopy, were as spiral shaped (BI26 and BI28) and Rectiflexibiles (BI02, BI21, BI24).

In the carbohydrate assimilation test, carbon sources such as glucose, arabinose, sucrose, xylose, inositol, raffinose and mannitol were utilized by the strains efficiently (Table 2). All the data obtained with regard to the physiological and biochemical properties of the isolates, therefore, strongly confirmed that the strains BI02, BI21, BI24, BI26 and BI28 belonged to the *Streptomyces* genus.

Table 2. Utilization of different carbon sources by *Streptomyces* spp. Isolates.

Carbon sources	Growth of *Streptomyces* spp. strains				
	BI02	BI21	BI24	BI26	BI28
D-glucose	+	+	+	+	+
L-arabinose	+	+	+	+	+
Sucrose	+	+	+	+	+
D-fructose	-	-	-	-	-
D-xylose	+	+	+	+	+
I-inositol	+	+	-	-	+
Raffinose	+	+	+	+	+
D-mannitol	+	+	+	+	+
Cellulose	-	-	-	-	-

Table 3. Elucidation of functional traits of antagonistic plant growth promoting rhizobacteria.

Isolates	Activity				
	Chitinase	Protease	Phosphate solubilization	Siderophore	Antimicrobial
BI01	+	-	-	-	-
BI02	+	-	+	+	+
BI12	-	-	-	-	-
BI13	+	-	+	-	-
BI14	-	+	-	+	-
BI15	+	+	+	+	-
BI16	-	-	+	-	-
BI21	+	-	+	+	+
BI24	+	+	+	+	+
BI25	-	-	-	+	-
BI26	+	+	+	+	+
BI27	-	+	-	+	-
BI28	+	-	+	+	+
BI29	+	-	+	-	-
BI30	-	+	+	-	-
BI32	-	+	-	-	-
BI34	-	-	-	+	-
BI35	-	-	-	-	-
BI38	-	-	+	-	-
BI41	+	-	-	-	-
BI42	-	-	-	+	-
BI43	-	-	+	-	-
BI45	+	-	-	-	-
BI46	-	-	+	-	-
Percentage (%)	45.83	29.17	54.17	45.83	20.83

- = Negative result.
+ = Positive result

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, 24 isolates were evaluated and 13 (54,17 %) isolates showed P-solubilizing activity in 7 days (Table 3). The five isolates BI02, BI21, BI24, BI26 and BI28 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 (Naik et al., 2008). The evaluated strains seem to possess P stabilizing in soil. The mechanisms of P stabilizing already elaborated previously (Pikovskaya; 1948; Richardson et al., 2009; Franco-Correa et al., 2010).

IAA production

The IAA production was by the development of pink color in ISP2 culture medium after the addition of salkowski reagent to the culture. Among 24 actinobacteria, 5 were able to produce high levels of IAA.

Interestingly, *Streptomyces* spp. strains BI24 produced highest amount of IAA as compared to the four other strains (Figure 3). Root exudates are important sources of natural tryptophan, which may enhance microbial biosynthesis of IAA (Khamna et al., 2009). The production threshold of IAA by such PGPR bio-inoculants will be critical in effecting beneficial root response (Kochar et al., 2011). Strains *Streptomyces* spp. and its IAA production could be recommended as useful bio-inoculants based on the plant growth parameters analysed.

Fungal cell wall-degrading enzymes and antagonistic substances production

Characters like cell wall degradation have been heavily reported as important mechanisms of antifungal action for various *Streptomyces* species (Froes et al., 2012; Sadeghy and Hatamy, 2014; Al-Askar et al., 2015; Sabaratnam and Traquair, 2015). 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 3). 11 (45.83%) rhizosphere strains showed fungal cell wall-degrading enzyme chitinase activity. Proteolytic activity was detected for 4 of 7 (29,17 %). In general, the higher chitinase activity was correlated with higher fungal inhibition. It has been reported that antifungal mechanism of antagonists has been attributed to the action of hydrolytic enzymes such as chitinase, (Quecine et al., 2008) and protease (Naing et al., 2014).

As a next step, we determined the contribution of siderophore production to antagonistic properties of the isolated *Streptomyces* spp. (Table 3). From 24 *Streptomyces* isolates, 11 (45.83%) formed halo zones on CAS agar media, indicating strong siderophore production. Among the five strains, BI02, BI21, BI24, BI26 and BI28 had relatively higher siderophore production. All the five *Streptomyces* spp. isolates produced siderophores which is a positive indication of iron chelating ability. Siderophore production by *Streptomyces* strains has been well documented (Jog et al., 2012). A similar observation has also been made by Kumar et al., (2011) for rhizobia. Siderophores production is primarily responsible for the stimulation of the root development (Walia et al., 2014).

Antagonisms

The actinobacteria were initially screened to determine their antimicrobial activity by following cross streak method. Actinobacteria strains exhibiting the ability to produce both clear zones of inhibition and metabolites against the tested pathogenic bacteria or fungi were considered antagonistic. The 24 *Streptomyces* spp. isolates were tested for the antimicrobial activity. Only five (20.83 %) BI02, BI21, BI24, BI26 and BI28 showed the strongest inhibitory activity against all tested microorganisms (Table 3) and indicating a good antimicrobial spectrum, with large inhibition zone (Figure 2).

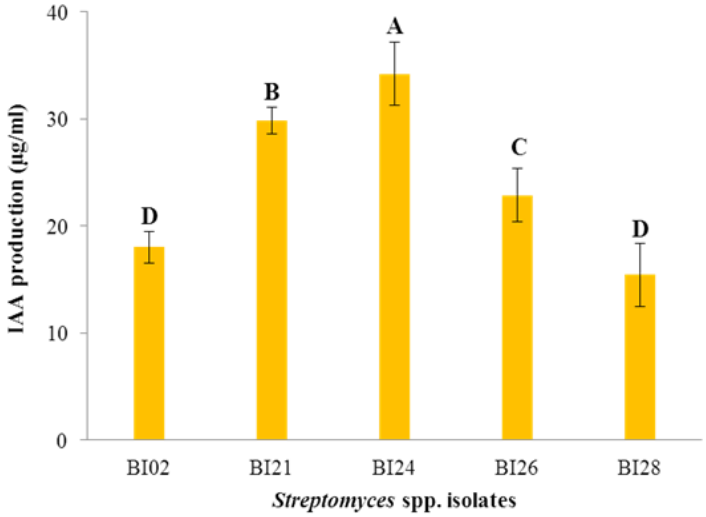

Fig. 3. Production of Indole acetic acid (IAA) by Streptomyces spp. The results are mean values of three data sets, (Duncan's test, $p < 0.05$), different letters on top of the histograms indicate significant differences.

Table 4. Individual analysis of *Streptomyces* spp. (BI02, BI21, BI24, BI26 and BI28) strains for their antagonistic property.

Strains	Diameter of inhibition zone (mm)*					
	Indicator strains					
	F. oxysporum f.sp.albedinis	*F. culmorum*	*M. ramannianus*	*B. subtilis*	*P. syringae*	*E. coli*
BI02	16.3 ± 3.2^{d}	24,3 ± 0,6^{b}	12.0 ± 2.0^{b}	19.3 ± 2.1^{c}	25.7 ± 4.0^{b}	5.5 ± 1.5^{d}
BI21	35.0 ± 3.0^{a}	31,7 ± 2,1^{a}	24.3 ± 2.1^{a}	7.0 ± 0.5^{d}	20.7 ± 1.2^{c}	12.3 ± 2.5^{c}
BI24	30.7 ± 1.2^{ba}	21,3 ± 1,5^{b}	10.3 ± 1.5^{b}	31.7 ± 2.5^{a}	32.0 ± 3.6^{a}	30.0 ± 2.0^{a}
BI26	23.3 ± 3.1^{c}	15,7 ± 2,1^{c}	13.7± 1.5^{b}	18.3 ± 1.5^{c}	11.0 ± 1.7^{d}	15.7 ± 2.1^{b}
BI28	27.3 ± 2.5^{bc}	18,0 ± 2.0^{c}	22.0 ± 2.0^{a}	23.7 ± 3.1^{b}	19.0 ± 1.0^{c}	28.7 ± 1.1^{a}

*: 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 ($p < 0.05$).

The highest inhibition was shown by *Streptomyces* spp. BI21 against *F. oxysporum* f.sp. *albedinis* (35 mm), *F. culmorum* (31.7 mm) and *M. ramannianus* (24.3 mm). *Streptomyces* spp. BI24 showed high activity against *B. subtilis* (31.7 mm), *P. syringae* (32 mm) and *E. coli* (30 mm). *Streptomyces* spp. BI28 showed high activity against *M. ramannianus* (22 mm) and *E. coli* (28.7 mm) (Table 4).

The broad-spectrum activity exhibited by some of the isolates is possibly due to the production of diverse antimicrobial compounds. The antagonism of *Streptomyces* observed in this study is in accordance with previous reports (Khamna et al., 2009; Kavitha et al., 2010). According to Kavitha et al. (2010), *Streptomyces* sp.TK-VL_333 showed antagonistic activity toward a variety of bacteria, yeast and filamentous fungi. Verma et al. (2009), observed that approximately 60 % of the isolated actinobacterias have 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 *Astragalus gombo* is a useful potential source for isolation of actinobacterias producers of biological active products.

Biocontrol agents

The biocontrol potential of five *Streptomyces* spp. was assessed according to their microbial degrading activity and activities of fungal cell wall degrading enzymes including protease and chitinase. The seeds of date palm infested with Foa and bacterized with strains BI28, BI26, BI24, BI21, or BI02 showed enhanced seed

germination as compared to the seeds inoculated with FAO alone and the seeds not inoculated (control) (Fig. 4). The strains BI28 and BI26 resulted in 93.33 % and 91.67 % seed germination respectively, while BI24 and BI21 resulted 88.33 %, as well as BI02 resulted 83.33 % seed germination as compared to the control (81.67%) or seeds inoculated with Foa (11.67 %). Kumar et al. (2011) also observed the enhanced seed germination of fenugreek (*Trigonella foenum-graecum* L.) seeds inoculated with rhizobia. Increased index of germination was observed from the palm date 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 (Li et al., 2011; Souaghi et al., 2015) and phytohormone (Li et al., 2011; Souaghi et al., 2015; Spaepen and Vanderleyden, 2011) by *Steptomyces* spp. strains, might be involved in increasing seed germination and controlling plant pathogenicity (Al-Askar et al., 2015; Sbaratnam and Traquair, 2015).

Based on controlled conditions test results, the five isolates displayed high biocontrol efficacy against fusarium wilt, spicily BI24 isolate. This may be attributed to the efficiency of BI24 to produce higher amount of antimicrobial compound which compared with other tested actinobacteria strain.

Effects of the five *Streptomyces* spp. stains (BI28, BI26, BI24, BI21 and BI02) on palm date growth were evaluated based on the increases in shoot height, shoot fresh weight, root fresh weight and root length of the plant. The measurements of growth parameters of palm date inoculated with Fao, treated with *Streptomyces* spp. or not and non-inoculated plants (control) are shown on Fig. 5.

Streptomyces spp. BI24 caused a significant increase in root length (Fig. 5a) and root fresh weight (Fig. 5b) compared to control or to the infested plants. Furthermore, all *Streptomyces* spp. caused significant increase in root length and fresh weight compared to infested plant with Foa. On the other hand, *Streptomyces* spp. BI24 and BI21 caused a significant increase in shoot height (Fig. 5c), whereas BI24, BI21 and BI26 increased significantly the shoot fresh weight (Figure 5d). Interestingly, plants treated with the *Streptomyces* spp. BI24 and BI21, showed significant both higher shoot height and fresh weight, compared even with the infested plants and no inoculated plants (control).

The role of *Streptomyces* in plant growth promotion is widely known. Many species of *Streptomyces* including *Streptomyces* spp. (Quecine et al., 2008; Fialho et al., 2010; Bubici et al., 2013), *Streptomyces goshikiensis* (Faheem et al., 2015), *Streptomyces aureoverticillatus* (Wang et al., 2015), *Streptomyces lydicus* (Li et al., 2015) and *Streptomyces sioyaensis* (Nakaew et al., 2015) are widely known for their biocontrol abilities as they produce several antibiotics and lytic enzymes, and supports plant growth promotion by production of phytohormones, solubilization of phosphate. In other hand, Haggag et al. (2015) reported that application each of *Bacillus pumilus* Bp3 *B. subtilis* Bs3, and *Pseudomonas fluorescens* Pf2 isolates in combination with pencycuron is a promising approach for the improvement of bean root rot control.

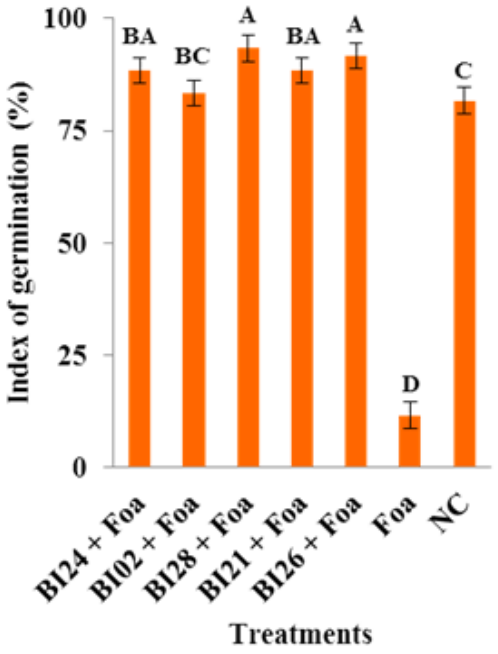

Fig. 4. Effect of *Streptomyces* spp. strains (BI24, BI02, BI28, BI21 and BI26) on the germination index of date palm (*Phoenix dactylifera* L.) inoculated with pathogenic fungus *Fusarium oxysporum* f.sp. *albedinis* (Foa). Duncan's test ($p < 0.05$) showed that the histograms using different letters were significantly different. Values are means of three independent experiments and error bars indicates standard deviation. NC (Negative control): no inoculated plants.

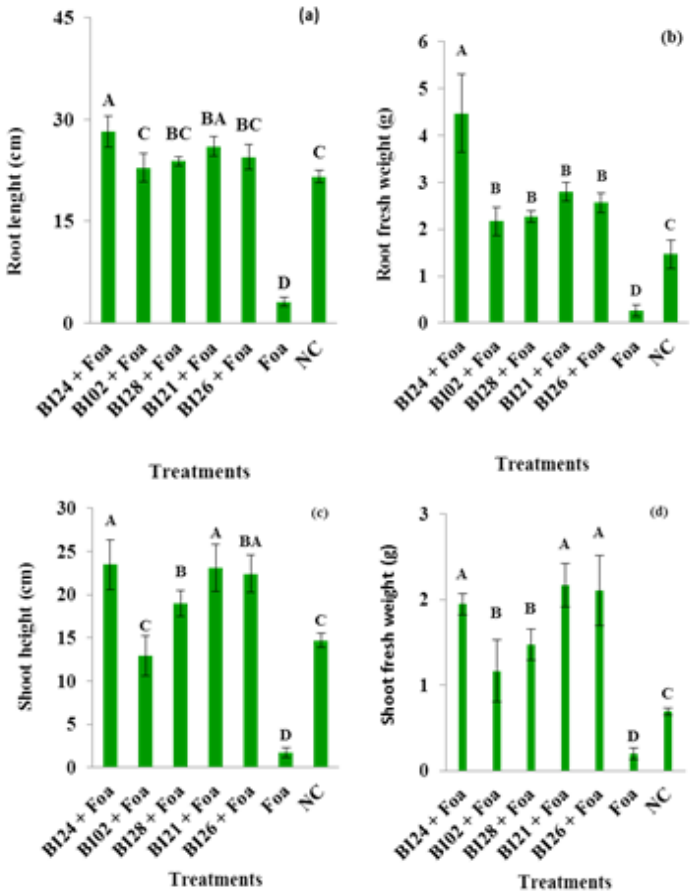

Fig. 5. Effect of *Strptomyces* spp. strains on the, root length (a), root fresh weight (b) shoot height (c) shoot fresh weight (d) of date palm (*Phoenix dactylifera* L.) inoculated with pathogenic fungus *Fusarium oxysporum* f.sp. *albedinis* (Foa). Duncan's test ($p < 0.05$) showed that the histograms using different letters were significantly different. Values are means of three independent experiments and error bars indicates standard deviation. NC (Negative control): Plants without *Streptomyces* spp. strains and Foa

Conclusion

From the results of this investigation, it can be concluded that these five *Streptomyces* spp. Isolates reveals the ability of promoting palm date plant growth, which probably because of the production of IAA, phosphate solubilization enzymes and siderophore. The isolates BI02, BI21, BI24, BI26 and BI28 were selected for their activity and their good antimicrobial spectrum against pathogenic microorganism, and identified as *Streptomyces* spp. by phenotypic characteristics. These results clearly suggest the possibility of using *Streptomyces* as biocontrol agent against bayoud disease for palm date, and demonstrates the high potential for plant growth promotion. Further study is required to demonstrate the mechanism of its action and evaluate the effect of the biocontrol potential against Fusarium wilt disease of palm date in field conditions.

Author contributions

All authors contributed equally in the study and preparation of article. All authors approved the final version of the manuscript for publication

References

Ait Kettout, T. and F. Rahmania. (2010). Identification par CG-SM de l'acide phénylacétique produit par *Fusarium oxysporum* f. sp. albedinis, agent causal du bayoud. *Comptes Rendus Biologies*. *333*: 808-813.

Al-Askar, A.A., Y.M. Rashad, E.E. Hafez, W.M. Abdulkhair, Z.A. Baka and K.M. Ghoneem. (2015). Characterization of alkaline protease produced by *Streptomyces*

griseorubens E44G and its possibility for controlling Rhizoctonia root rot disease of corn. *Biotechnology & Biotechnological Equipment*. *29*: 457- 462.

Bhattacharya, A., S. Chandra and S. Barik. (2009). Lipase and protease producing microbes from the environment of sugar beet field. *Indian Journal of Agricultural Biochemistry*. *22*: 26-30.

Boufis, N., M. Khelifi-Slaoui, Z. Djillali, D. Zaoui, A. Morsli, M.A. Bernards, A. Makhzum and L. Khelifi. (2014). Effects of growth regulators and types of culture media on somatic embryogenesis in date palm (*Phoenix dactylifera* L. cv. Degla Beida). *Scientia Horticulturae*. *172*(9): 135-142.

Bouguedoura, N., M. Bennaceur, S. Babahani and S.E. Benziouche. (2015). Date Palm Status and Perspective in Algeria Chapter 4 in: J.M. Al-Khayri et al. (eds.), Date Palm Genetic Resources and Utilization: Volume 1: Africa and the Americas, © Springer, pp.125-168.

Brac de la Perri, R.A., H. Ami and N. Bounaga. (1995). Prospects for integrated control of 'bayoud' (Fusarium wilt of the date palm) in Algerian plantations. *Crop Protection*. *14*(3): 227-235.

Bubici, G., A.D. Marsico, M. D'Amico, M. Amenduni and M. Cirull. (2013). Evaluation of. for the biological control of corky root of tomato and Verticillium wilt of eggplant. *Applied Soil Ecology*. *72*: 128-134.

Chauhan, H., D.J. Bagyaraj, G. Selvakumar and S.P. Sundaram. (2015). Novel plant growth promoting rhizobacteria—Prospects and potential. *Applied Soil Ecology*. *95*: 38-53.

de Lima Procópio, R.E., I.R. da Silva, M.K. Martins, J.L. de Azevedo and J.M. de Araújo. (2012). Antibiotics produced by *Streptomyces*. *Brazilian Journal of Infectious Diseases*. *16*(5):466-471.

El Hassni, M., A. El Hadrami, F. Daayf, M. Chérif, E. Ait Barka and I. EL Hadrami. (2007). Biological control of bayoud disease in date palm: Selection of microorganisms inhibiting the causal agent and inducing defense reactions. *Environmental and Experimental Botany*. *59:* 224–234.

Faheem, M., W. Raza, W. Zhong, Z. Nan, Q. Shen and Y. Xu. (2015). Evaluation of the biocontrol potential of Streptomyces goshikiensis YCXU against *Fusarium oxysporum* f.sp. niveum. *Biological Control*. *81:* 101-110.

Fialho de Oliveira, M., M. Germano da Silva and S.T. Van Der Sand. (2010). Anti-phytopathogen potential of endophytic actinobacteria isolated from tomato plants (*Lycopersicon esculentum*) in southern Brazil, and characterization of *Streptomyces* sp. R18(6), a potential biocontrol agent. *Research in Microbiology*. *161*(7): 565-572.

Franco-Correa, M., A. Quintana, C. Duque, C. Suarez, M.X. Rodríguez and J-M. Barea. (2010). Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. *Applied Soil Ecology*. *45*: 209-217.

Ghadbane, M., Medjekal, S., Belhadj, H., Bounar, R., Khellaf, R., Benderradji, L., Smaili, T., & Harzallah, D. (2015). Biocontrol of Chickpea wilt Disease by Streptomyces Sp. Isolated from the Rhizosphere of *Ononis angustissima* Lam. *World Applied Sciences Journal, 33*(9), 1414-1427,

Ghadbane, M., S. Medjekal, H. Belhadj, R. Bounar, R. Khellaf, L. Benderradji, T. Smaili and D. Harzallah. (2015). Biocontrol of chickpea wilt disease by *Streptomyces* sp. isolated from the rhizosphere of *Ononis angustissima* Lam. *World Applied Sciences Journal*. *33* (9): 1414-1427.

Gupta, G., S.S. Parihar, N.K. Ahirwar, S.K. Snehi and V. Singh. (2015). Plant Growth Promoting Rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture. *Journal of Microbial and Biochemical Technology*. *7*: 96-102.

Gupta, R., R.K. Saxena, P. Chaturvedi and J.S. Virdi. (1995). Chitinase production by *Streptomyces viridificans*: its potential in fungal cell wall lysis. *Journal of Applied Microbiology*. *78:* 378-383.

Haggag, H.E.K., I.E. Elshahawy and H. Abd-El-Khair. (2015). Antagonistic activity of Bacillus and Pseudomonas isolates alone or in combination with fungicides against some soil borne plant pathogens under laboratory and greenhouse conditions. *Middle-East Journal of Scientific Research*. *23* (10): 2354-2365.

Idder, M.A., H. Ighili, B. Mitiche and H. Chenchouni. (2015). Influence of date fruit biochemical characteristics on damage rates caused by the carob moth (*Ectomyelois ceratoniae*) in Saharan oases of Algeria. *Scientia Horticulturae. 190*: 57-63.

Jacobson, E., W.C. Grauville and C.E. Fogs. (1958). Color Harmony Manual, 4th ed. Chicago: Container Corporation of America.

Jog, R., G. Nareshkumar and S. Rajkumar. (2012). Plant growth promoting potential and soil enzyme production of the most abundant *Streptomyces* spp. from wheat rhizosphere. *Journal of Applied Microbiology. 113*: 1154-1164.

Karlidag, H., A. Esitken, M. Turan and F Sahin. (2007). Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of apple. *Scientia Horticulturae. 114*: 16-20.

Karthik, N., P. Binod and A Pandey. (2015). Purification and characterization of an acidic and antifungal chitinase produced by a *Streptomyces* sp. *Bioresource Technology. 188*: 195-201.

Kaur, T., D. Sharma, A. Kaur and R.K. Manhas. (2013). Antagonistic and plant growth promoting activities of endophytic and soil actinomycetes. *Archives of Phytopathology and Plant Protection. 46*: 1756-1768.

Kavitha, A., P. Prabhakar, M. Vijayalakshmi and Y. Venkateswarlu. (2010). Purification and biological evaluation of the metabolites produced by *Streptomyces* sp.TK-VL_333. *Research in Microbiology. 161*(5): 335-345.

Kawase, T., A. Saito, T. Sato, R. Kanai, T. Fujii, N. Nikaidou, K. Miyashita and T. Watanabe. (2004). Distribution and phylogenetic analysis of family 19 chitinases in Actinobacteria. *Applied and Environmental Microbiology. 70*(2): 1135-1144.

Khamna, S., A. Yokota aand S. Lumyong. (2009). Actinobacteria isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. *World Journal of Microbiology and Biotechnology. 25*(4): 649-655.

Khieu, T-N., M-J. Liu, S. Nimaichand, N-T. Quach, S. Chu-Ky, Q-T. Phi, T-T. Vu, T-D. Nguyen, Z. Xiong, D.M. Prabhu and W-J. Li. (2015). Characterization and evaluation of antimicrobial and cytotoxic effects of Streptomyces sp. HUST012 isolated from medicinal plant *Dracaena cochinchinensis* Lour. *Frontiers in Microbiology. 6*:574.

Kochar, M., A. Upadhyay and S Srivastava. (2011). Indole-3-acetic acid biosynthesis in the biocontrol strain *Pseudomonas fluorescens* Psd and plant growth regulation by hormone overexpression. *Research in Microbiology. 162*: 426-435.

Kriaa, W., B. Sghaier-Hammami, F. Masmoudi-Allouche, R. Benjemaa-Masmoudi and N. Drira. (2012). The date palm (*Phoenix dactylifera* L.) micropropagation using completely mature female flowers. *Comptes Rendus Biologies. 335*(3): 194-204.

Kumar, H., R.C. Dubey and D.K. Maheshwari. (2011). Effect of plant growth promoting rhizobia on seed germination, growth promotion and suppression of Fusarium wilt of fenugreek (*Trigonella foenum-graecum* L.). *Crop Protection. 30*: 1396-1403.

Kumar, P.S., V. Duraipandiyan and S. Ignacimuthu. (2014). Isolation, screening and partial purification of antimicrobial antibiotics from soil *Streptomyces* sp. SCA 7. *The Kaohsiung Journal of Medical Sciences. 30*(9): 435-446.

Lee, B.D., S. Dutta, H. Ryu, S-J. Yoo, D-S. Suh and K. (2015). Park. Induction of systemic resistance in *Panax ginseng* against *Phytophthora cactorum* by native *Bacillus amyloliquefaciens* HK34. *Journal of Ginseng Research. 39*(3): 213-220.

Li, J., W. Liu, L. Luo, D. Dong, T. Liu, T. Zhang, C. Lu, D. Liu, D. Zhang and H. Wu. (2015). Expression of *Paenibacillus polymyxa* β-1,3-1,4-glucanase in Streptomyces lydicus A01 improves its biocontrol effect against *Botrytis cinerea*. *Biological Control. 90:* 141-147.

Li, Q., Y. Jiang, P. Ning, L. Zheng, J. Huang, G. Li, D. Jiang and T. Hsiang. (2011). Suppression of *Magnaporthe oryzae* by culture filtrates of Streptomyces globisporus JK-1. *Biological Control. 58(2):* 139-148.

Mathivanan, S., Chidambaram, AL. A., G. Amalan Robert and Kalaikandhan, R. (2017). Impact of PGPR inoculation on photosynthetic pigment and protein

contents in *Arachis hypogaea* L.. *Journal of Scientific Agriculture, 1*, 29-36. doi:10.25081/jsa.2017.v1i0.24

Murashige, T and F Skoog. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiologia Plantarum. 15(3):* 473-492.

Naik, P.R., G. Raman, K.B. Narayanan and N. Sakthivel. (2008). Assessment of genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads isolated from rhizospheric soil. *BMC Microbiology. 8:* 230.

Naing, K.W., M. Anees, S.J. Kim, Y. Nam, Y.C and Kim, K.Y. Kim. (2014). Characterization of antifungal activity of *Paenibacillus ehimensis* KWN38 against soil borne phytopathogenic fungi belonging to various taxonomic groups. *Annals of Microbiology. 64:* 55-63.

Nakaew, N., C. Rangjaroen and R. Sungthong. (2015). Utilization of rhizospheric *Streptomyces* for biological control of *Rigidoporus* sp. causing white root disease in rubber tree. *European Journal of Plant Pathology. 142:* 93-105.

Pikovskaya, R.I. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya. 17*: 363-370.

Piromyou, P., B. Buranabanyat, P. Tantasawat, P. Tittabutr, N. Boonkerd and N. Teaumroong. (2011). Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand. *European Journal of Soil Biology. 47:* 44-54.

Quecine, M.C., W.L. Araujo, J. Marcon, C.S. Gai, J.L. Azevedo and A.A. Pizzirani-Kleiner. (2008). Chitinolytic activity of endophytic *Streptomyces* and potential for biocontrol. *Letters in Applied Microbiology. 47*: 486-491.

Richardson, A.E., J.M. Barea, A.M. McNeill and C. Prigent-Combaret. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and Soil. 321:* 305-339.

Sabaratnam, S. and J.A. Traquair. (2015). Mechanism of antagonism by *Streptomyces griseocarneus* (strain Di944) against fungal pathogens of greenhouse-grown tomato transplants. *Canadian Journal of Plant Pathology. 37:* 197-211.

Sadeghy, B. and N Hatamy. (2014). Isolation and investigation of extracellular metabolites of *Streptomyces* sp. for biological control of *Fusarium subglutinans*, the causal agent of wilting cucumber greenhouse. *Archives of Phytopathology and Plant Protection. 47*: 171-177.

Sathiyabama, M. and RE Charles. (2015). Fungal cell wall polymer based nanoparticles in protection of tomato plants from wilt disease caused by *Fusarium oxysporum* f.sp. lycopersici. *Carbohydrate Polymers. 133*: 400-407.

Schwyn, B. and J.B. Neilands. (1987). Universal chemical assay for the detection and determination of siderophores. *Annals of Biochemistry. 160*: 47-56.

Shirling, E.B. and D. Gottlieb. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology. 16*(3): 313-340.

Simonettia, E., N.P. Viso, M. Montecchia, C. Zillid, K. Balestrassec and M. (2015). Carmona. Evaluation of native bacteria and manganese phosphite for alternative control of charcoal root rot of soybean. *Microbiological Research. 180*: 40-48.

Sowndhararajan, K., S. Marimuthu and S. Manian. (2012). Biocontrol potential of phylloplane bacterium *Ochrobactrum anthropi* BMO-111 against blister blight disease of tea. *Journal of Applied Microbiology. 114*: 209-218.

Spaepen, S. and J. Vanderleyden. (2011). Auxin and plant-microbe interactions. *Cold Spring Harb Perspect Biol. 3(*4): a001438.

Subramani, R. and W. Aalbersberg. (2015). Marine actinomycetes: an ongoing source of novel bioactive metabolites. *Microbiology Research. 167*(10): 571-580.

Verma, V.C., S.K. Gond, A. Kumar, A. Mishra, R.N. Kharwar and A.C. Gange. (2009). Endophytic actinomycetes from *Azadirachta indica* A. Juss.: isolation, diversity, and anti-microbial activity. *Microbial Ecology. 57*(4): 749-756.

Walia, A., P. Mehta, A. Chauhan and C.K. Shirkot. (2014). Effect of *Bacillus subtilis* strainCKT1 as inoculum on growth of tomato seedlings under net house conditions. *Proc. Natl. Acad. Sci., India Sect. B: Biol. Sci. 84(1):* 145-155.

Wang, L., M. Xing, R. Di and Y. Luo. (2015). Isolation, identification and antifungal activities of *Streptomyces aureoverticillatus* HN6. *Journal of Plant Pathology and Microbiology*. *6*(6): 1-5.

Williams, S.T., M. Goodfellow and G. Alderson. (1989). Genus Streptomyces Waksman and Henrici 1943. 339AL. In: Bergeys Manual of Systematic Bacteriology, Williams, S.T., M.E. Sharpe and J.G. Holt (Eds.). Williams and Wilkins Co., Baltimore. ISBN: 0-683-09061-5.

Wu, Y., C. Zhao, J. Farmer and J. Sun. (2015). Effects of bio-organic fertilizer on pepper growth and Fusarium wilt biocontrol. *Scientia Horticulturae*. *193,* 114-120