

### Selection of compost-derived actinomycetes with plant-growth promoting and tomato stem rot biocontrol potentialities

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#### Abstract

Seventeen actinomycetes isolates, recovered from 2 composts, were screened for their ability to promote the growth of tomato seedlings and to suppress stem rot disease caused by Sclerotium rolfsii. Tomato cv. Rio Grande seedlings inoculated with S. rolfsii and treated with A2-3, A3-3, A4-3, A5-3, A8-3, A9-3, A1-4, A2-4, A3-4, A4-4, A6-4, and A10-4 actinobacterial isolates showed 23.3-70% less disease severity than the inoculated and untreated controls. A3-3, A2-4, and A4-4 based treatments applied to S. rolfsü-infected tomato seedlings had significantly enhanced all growth parameters as compared to control. The recorded increments were estimated at 35.52-66.6% for height, 37.4-53.4% for the stem diameter, 38.5-95.6% for the aerial part dry weight, and 81.8-151% for the root dry weight. Treatments with A3-3 and A4-4 isolates had increased the majority of tomato growth parameters by 15.8-56.5% over the pathogen-free control. Tomato seedlings treated with A4-3 and A1-4 isolates showed between 35.2-22.8% and 42.3-43.3% higher aerial part dry weight and root dry weight, respectively, as compared to pathogen-free and untreated control. This investigation demonstrated that the tested composts can be explored as potential sources for the isolation of actinomycetes acting as biocontrol and bio-fertilizing agents.

Keywords: actinobacteria, antifungal activity, growth enhancement, Sclerotium rolfsii, Solanum lycopersicum.



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#### 1. Introduction

Composting is defined as a biochemical process managed under principally thermophilic and aerobic conditions by active microorganisms to produce a renewable organic resource (Bohacz, 2018). This process is a sustainable option for recycling various agro-industrial wastes, on small or large scale, to obtain a mature and stable organic matter (Scotti et al., 2016). The final product, i.e., compost, can be used to improve soil physicochemical and microbiological properties and suppress various soilborne diseases (Pane et al., 2019; Stavi et al., 2016). These effects are partly attributed to their associated beneficial microorganisms and bioactive metabolites (Hadar & Papadopoulou, 2012). Thus, composts are becoming an alternative tool for the extensive use of synthetic inputs in cropping systems and are deemed safer for the environment and human health (Coventry et al., 2006). In the past decades, the added value of composts attributed to their disease-suppressive effects have been extensively demonstrated against soilborne pathogens such various as Rhizoctonia solani, Verticillium dahliae, Fusarium spp., Sclerotinia spp., Phytophthora spp., Pythium spp., and Thielaviopsis sp. causing plant wilting, damping-off and decaying in many important crops (Tubeileh & Stephenson, 2020; Pane et al., 2013; Alfano et al., 2011; Bonanomi et al., 2007). The compost disease-suppressive potential is mainly related to the biological activity of its associated microbiota, which interacts with the soil organic matter and the host plant by regulating the microbial communities in the rhizosphere (Hadar & Papadopoulou, 2012; Manici et al., 2004), and to its capacity to improve plant nutrition and growth (Martin, 2015). Among the widely documented compost-associated microbial agents, bacteria, actinomycetes, and fungi were the focal driving forces for plant diseases suppression (Coelho et al., 2020; Joshi et al., 2009). These microbial agents acted against target pathogens through antibiosis,

hyperparasitism, and competition for space and nutrients (Larkin & Tavantzis, 2013). Actinomycetes, gram-positive bacteria, and members of the Actinobacteria group are well known as secondary metabolites producers and are widely explored for various agricultural features (Qin et al., 2011). Actinomycetes have been recovered from diverse natural environments such as rhizospheric soil. and healthy plant composts, tissues. Commercial bio-molecules mostly are produced by Streptomyces, Saccharopolyspora, Micromonospora, Amycolatopsis, and Actinoplanes (Palla et al., 2018). These microbial agents can improve plant growth and support its establishment even under stress conditions (Srivastava et al., 2015; Hamdali et 2008). Moreover, their antagonistic al., potential against phyto-pathogenic organisms was demonstrated (Nurkanto & Julistiono, 2014; Anouar et al., 2012). They can protect roots by inhibiting the fungal pathogen development mostly through the production of antifungal compounds or cell wall degrading enzymes (Bhatti et al., 2017). Sclerotium rolfsii Sacc. is a soilborne pathogen that affects a wide host range of over 500 dicotyledonous and monocotyledonous plant species (Sun et al., 2020; Punja, 1985; Aycock, 1966). Infection by this pathogen may occur at all growing stages and lower stems at or near the soil surface by forming water-soaked lesions. These lesions spread quickly to girdle stems where white mycelial mats may be observed on the infected plant tissues. Severely infected plants may wilt thus leading to partial or total yield loss (Sun et al., 2020; Kator et al., 2015; Fery & Dukes, 2002). Stem rot diseases are usually managed using chemical fungicides such as carboxin, carbendazim, benomyl, propiconazole, methyl thiophanate, and oxycarboxin (Sridharan et al., 2020). Nevertheless, the hazardous use of these chemicals represents a severe threat to the environment, food safety, and human health (Sridharan et al., 2020). Biological control of soilborne phytopathogens is of increased where various microorganisms interest

recovered from diverse ecological niches are largely explored for sustainable management of stem rot disease (Singh et al., 2013). Among the explored microorganisms, actinomycetes are considered as promising potential candidates for the suppression of S. rolfsii (Anusree & Bhai, 2017). Therefore, the main objectives of this investigation are: (1) to isolate actinomycetes associated with two selected composts; (2) to evaluate their ability to promote the growth of tomato seedlings and to control stem rot disease.

#### 2. Materials and methods

#### 2.1 Pathogen growth conditions

*S. rolfsii* isolate Sr<sub>1</sub>, used in the current study, was originally recovered from potato plants showing typical stem rot symptoms. Identification and pathogenicity of this isolate were previously investigated (Ayed et al., 2018a; Daami-Remadi et al., 2010). The pathogen was grown on Potato Dextrose Agar (PDA) medium for 15 days at 30 °C, in the dark, before used.

#### 2.2 Composts preparation

Bioassays were carried out using 2 mature composts. The first one  $(C_3)$  are composed by a 70% of cattle and 25% of chicken manures mixture associated with 5% green waste, the second one  $(C_4)$  are issued from 70% of cattle and 25% of sheep manures mixed with 5% of olive-mill solid waste. The composting system was carried out in parallel open-windrows in the following dimensions: height  $1.5 \text{ m} \times \text{base}$ width 2.0 m  $\times$  length 10 m. The compost mass was mechanically homogenized and the humidity of the mass during fermentation was maintained using water at an optimal level (60-70%) for the composting process for 8 months. These two locally produced composts and their teas were screened for their plant growthpromoting potential and for their ability to control stem rot disease on tomato seedlings (Ayed et al., 2018b; 2018c).

## 2.3 Isolation of compost-associated actinomycetes

A sample of 10 g of air-dried compost was suspended into a 90 ml volume of sterile distilled water (SDW) in a 250 ml flask, and shaken for 30 min at 200 rpm. Serial dilution of compost was carried out, and then a sample of 100 µl was spread onto Petri-dish containing Actinomycetes Isolation Agar medium amended with 500 µg/ml of streptomycin sulfate, 100 µg/ml of chloramphenicol, 50 µg/ml of ampicillin, and 100 µg/ml of cycloheximide (Joshi et al., 2009). Plates were maintained at  $28 \pm 2$  °C for 14-21 days, in darkness. Developed colonies showing distinct macro-morphological traits were isolated separately on Yeast Malt Agar (ISP-2), and stored in glycerol (20%) at -20 °C for further use (Himaman et al., 2016).

## 2.4 Morphological characterization of the actinomycete isolates

Obtained isolates were grown in ISP-2 medium at  $28 \pm 2^{\circ}$  C for 10 days. Actinobacterial colonies were characterized based on the colony appearance, the type of areal hyphae, and the growth of vegetative hyphae. The color of diffusible pigment production was visually estimated with the help of RHS-color code (RHS color chart, Fifth Edition-Royal Horticultural Society) (Anusree & Bhai, 2017).

#### 2.5 Screening for actinomycetes growthpromoting ability

Actinomycete isolates were assessed for their ability to promote the tomato seedlings growth. Tomato cv. Rio Grande seeds were surface-sterilized by immersing in 5% of sodium hypochlorite (NaOCl) for 3 min, then rinsed 6 times with SDW and air-dried (Aydi Ben Abdallah et al., 2018). Disinfected seeds were soaked for 30 min into actinobacterial cell suspensions ( $\sim 10^8$  CFU/ml), prepared in ISP-2 liquid cultures incubated at  $28 \pm 2$  °C for 48 h. They were sown in alveolated trays (4 cm  $\times 4 \text{ cm} \times 4 \text{ cm}$ ) filled with commercialized peat (Klasmann-Deilmann, **Bio-Substrate**, Germany), and watered regularly with tap water. A volume of 10 ml of each actinobacterial suspension was applied per alveolus by substrate drench immediately, 15 days, and 30 days post-sowing. Controls were soaked in SDW and watered later regularly with tap water. Ten seedlings were used for each treatment. The bioassay was carried out under plastic tunnels at 26-32 °C with 12 h (light) / 12 h (dark) photoperiod and 60% air relative humidity. At 35 days post-sowing, tomato seedlings were uprooted and washed for removing adhering peat. Different growth parameters were recorded (plant height, stem diameter, aerial part, and root dry weights). Data were analyzed according to a completely randomized design. The whole experiment was repeated twice.

## **2.6 Screening for actinomycetes stem rot suppression ability**

Tomato cv. Rio Grande seeds, disinfected as detailed above, were soaked for 30 min into a water cell suspension  $(10^8 \text{ CFU/ml})$  of collected actinomycetes isolates and sown in alveolated trays filled with commercialized peat (Aydi Ben Abdallah et al., 2018). All tested treatments were applied directly, 15 days and 30 days post-sowing (10 ml per alveolus per individual treatment). Challenge inoculation with *S. rolfsii* was performed as substrate drench with 20 ml of a mixture of mycelium and sclerotia ( $\approx 25-30$  sclerotia per

100 ml) 21 days post-sowing (Daami-Remadi et al., 2012). Uninoculated and inoculated controls were watered with the same volume of SDW and watered later regularly with tap water. The bioassay was carried out using ten seedlings per individual treatment and maintained under the same greenhouse conditions as indicated above. At 35 days postsowing, disease severity was recorded based on a 1-5 scale (De Curtis et al., 2010), where 1 = no stem lesion, 2 = lesions girdled  $\leq$  25% of the stem circumference, 3 = lesions girdled 26-50% of the stem circumference, 4 = lesions girdled > 51% of the stem circumference and 5 = stem completely girdled. Plant height, stem diameter, root, and aerial part dry weights were also noted. Data were analyzed according to a completely randomized design. The whole experiment was repeated twice.

#### 2.7 Statistical analysis

The data were statistically analyzed by analysis of variance (ANOVA) with the statistical package SPSS software (Version 20) and subjected to mean separation by Duncan Multiple Range test (at  $P \le 0.05$ ). Correlations between disease severity and seedling growth parameters were performed using bivariate Pearson's test at  $P \le 0.05$ .

#### 3. Results

# **3.1 Isolation of compost-associated actinomycetes and their morphological characterization**

A collection of 17 actinomycetes isolates, exhibiting diversity in their macromorphological traits, was recovered from the 2 composts  $C_3$  and  $C_4$ . The color of aerial mycelium of actinomycetes isolates varied from white to brownish-black, whereas the color of submerged mycelium varied from white to black. Colonies were circular or

irregular, raised or flat or punctiform, and entire or undulate (Table 1).

Table 1: Cultural characteristics of actinomycetes isolates on ISP-2 medium after incubation at  $28 \pm 2$  °C for 10 days.

Isolates	Composts	Color of aerial mycelium	Color of submerged mycelium	Colonies characteristics
A1-3	C <sub>3</sub>	White	White	Circular, raised, entire
A2-3	C <sub>3</sub>	Brownish-black	Brown	Circular, flat, entire
A3-3	C <sub>3</sub>	Brownish-black	Brown	Irregular, raised, undulate
A4-3	C <sub>3</sub>	Brownish-black	Brown	Circular, flat, punctiform
A5-3	C <sub>3</sub>	Greyed-brown	Brown	Irregular, raised, undulate
A 8-3	C <sub>3</sub>	Greyed-white	White	Circular, raised, entire
A9-3	C <sub>3</sub>	White	White	Circular, flat, punctiform
A10-3	C <sub>3</sub>	Greyed-black	Black	Circular, punctiform, raised
A1-4	$C_4$	White	White	Circular, punctiform, entire
A2-4	$C_4$	White	Greyed-yellow	Circular, flat, entire
A3-4	$C_4$	Greyed-brown	Greyed-brown	Irregular, raised, undulate
A4-4	$C_4$	Greyed-brown	Greyed-brown	Irregular, raised, undulate
A5-4	$C_4$	White	Greyed -white	Circular, raised, entire
A6-4	$C_4$	Greyed-green	Green	Circular, flat, entire
A7-4	$C_4$	White	White	Circular, raised, entire
A8-4	$C_4$	Greyed-white	White	Circular, raised, entire
A10-4	$C_4$	Grey	Greyed-white	Circular, raised, entire

#### **3.2 Disease suppression ability of compost**associated actinomycetes

ANOVA analysis revealed that stem rot severity, noted after 35 days post-sowing and 14 days post-inoculation, varied significantly depending on the tested actinobacterial treatments. As shown in Figure (1), a significant decrease in disease severity by 23.33 to 70% over pathogen-inoculated and untreated control was noted on tomato seedlings infected with *S. rolfsii* and treated with A2-3, A3-3, A4-3, A5-3, A8-3, A9-3, A1-

4, A2-4, A3-4, A4-4, A6-4, and A10-4 isolates. Tomato seedlings inoculated by the pathogen and treated with these isolates showed a significantly disease severity similar to the pathogen-free and untreated ones. The highest decreases in stem rot severity, of about 56.67 and 70%, were noted on tomato seedlings treated with A9-3 and A4-4 isolates, respectively. However, no disease-suppressive effect, as compared to the inoculated and untreated control, was noted on those treated with A1-3, A10-3, A5-4, A7-4, and A8-4 isolates.



Figure 1: Effect of compost-associated actinomycetes isolates on stem rot severity noted after 35 days post-sowing on *Sclerotium rolfsii*-inoculated tomato cv. Rio Grande seedlings as compared to the untreated controls. UC: Uninoculated and untreated control; IC: *S. rolfsii*-inoculated and untreated control. Results are presented as means  $\pm$  SE (n = 10,  $P \le 0.05$ ). Bars sharing the same letter are not significantly different according to Duncan's Multiple Range test (at  $P \le 0.05$ ).

#### **3.3** Growth-promoting ability of compostassociated actinomycetes on *Sclerotium rolfsü-*inoculated tomato seedlings

Growth parameters of tomato seedlings, noted after 35 days post-sowing and 14 days post-inoculation with *S. rolfsii*, varied significantly (at  $P \le 0.05$ ) depending on tested biological treatments. As illustrated in Table (2), treatments with A2-3, A3-3, A4-3, A5-3, A8-3, A9-3, A10-3, A1-4, A2-4, A3-4, A4-4, A5-4-, and A6-4 isolates significantly improved the seedling height by 21.1 to 66.6% over

pathogen-inoculated and untreated control, where the highest increment was induced by A4-4 treatment. Furthermore, a significant enhancement in the stem diameter, by 37.4 to 62.2% over pathogen-inoculated control, was recorded on tomato seedlings treated with A2-3, A3-3, A2-4, A3-4, and A4-4 isolates. A3-3 and A4-4 based treatments exhibited the highest growth-promoting effect based on the aerial part dry weight which was significantly improved by 95.6% and 66.2%, respectively, over the pathogen-inoculated and untreated control.

Table 2: Growth-promoting potential of compost-associated actinomycete isolates noted 35 days post-sowing
on tomato cv. Rio Grande seedlings inoculated with Sclerotium rolfsii as compared to the untreated controls.

Biological treatments	Plant height (mm)	Stem diameter (mm)	Aerial part dry weight (mg)	Root dry weight (mg)
UC	$81.5\pm1.35~b$	$2.28 \pm 0.1 \text{ a}$	189.87 ± 7.10 bcdef	26 ± 1.13 cdefg
IC	59.12 ± 1.17 de	$1.38 \pm 0.11 \text{ ef}$	142.25 ± 13.91 fg	$17.87 \pm 1.25$ fgh
A1-3	$39.75 \pm 5.28 \text{ f}$	$0.44 \pm 0.24$ g	58.37 ± 23.03 h	$11 \pm 1.88 \text{ h}$
A2-3	$73 \pm 6.16$ bc	$2.24 \pm 0.23$ a	$145.75 \pm 8.81 \text{ efg}$	$18.37 \pm 0.71$ fgh
A3-3	$80.12 \pm 6.96 \text{ b}$	$2.03 \pm 0.21$ abc	278.25 ± 17.78 a	39.37 ± 5.08 ab
A4-3	$82.25\pm5.43~b$	$1.77 \pm 0.16$ bcde	$214.62 \pm 17.86 \text{ bc}$	36.37 ± 4.97 abc
A5-3	$81.75 \pm 2.39 \text{ b}$	$1.57 \pm 0.11$ cde	176.75 ± 10.71 cdef	$24.37 \pm 0.98 \text{ defg}$
A8-3	$73.12 \pm 2.53$ bc	$1.78 \pm 0.13$ bcde	$184.75 \pm 17.60$ cdef	$30.62 \pm 6.61$ bcde
A9-3	$72.37 \pm 2.8 \text{ bc}$	$1.65 \pm 0.66$ cde	$146.5 \pm 11.25 \text{ efg}$	$19.75 \pm 0.90 \text{ efgh}$
A10-3	$73 \pm 2.1$ bc	$1.76 \pm 0.14$ bcde	169.37 ± 15.82 cdefg	$25 \pm 1.80 \text{ defg}$
A1-4	$76.37 \pm 5.53 \text{ bc}$	$1.65 \pm 0.20$ cde	$202.5 \pm 16.86$ bcd	$27.87 \pm 3.60$ cdef
A2-4	$80.5\pm3.9~b$	$2.12 \pm 0.08 \text{ ab}$	$197 \pm 11.27$ bcd	$32.5 \pm 3.02$ bcd
A3-4	$80.37 \pm 4.23 \text{ b}$	$2.02 \pm 0.09$ abc	$208.12 \pm 10.34$ bc	$24 \pm 2.15$ defg
A4-4	98.5 ± 3.57 a	$1.9 \pm 0.07 \text{ abcd}$	$236.37 \pm 18.32$ ab	44.87 ± 6.16 a
A5-4	$73.62 \pm 3.8 \text{ bc}$	$1.63 \pm 0.08$ cde	$195 \pm 20.54$ bcde	$36.25 \pm 6.39$ abc
A6-4	$71.62 \pm 1.77$ bc	$1.69 \pm 0.09$ bcde	184.37 ± 3.73 cdef	$27.37 \pm 2.07 \text{ cdef}$
A7-4	$49.12 \pm 0.55$ ef	$1.14\pm0.08~f$	$140.37 \pm 4.66 \text{ fg}$	$22 \pm 2.36$ defgh
A8-4	$64.62 \pm 4.5 \text{ cd}$	$1.53 \pm 0.19 \text{ def}$	127.75 ± 25.73 g	$16.37 \pm 3.13$ fgh
A10-4	50.87 ± 2.71 ef	$0.34 \pm 0.03$ g	$153.37 \pm 5.55 \text{ defg}$	$14.62 \pm 1.89$ gh

UC: Uninoculated and untreated control; IC: *S. rolfsii*-inoculated and untreated control. Results are presented as means  $\pm$  SE (n = 10,  $P \le 0.05$ ). For each column, values followed by the same letter are not significantly different according to Duncan's Multiple Range test (at  $P \le 0.05$ ).

Treatments with A4-3, A1-4, A2-4, A3-4, and A5-4 isolates led to a significant increment in this parameter by 37.1-50.9%. As for their effects on the root dry weight, A3-3, A4-3, A4-4, and A5-4 isolates had significantly improved this parameter by 102.8-151.1% over pathogen-inoculated and untreated control. It should be also highlighted that A8-3 and A2-4 based treatments led to a 71.3% and 81.8% increase in the root dry weight over control, respectively. However, A1-3, A7-4, A8-4, and A10-4 isolates had no positive

effects based on all studied growth parameters.

## **3.4** Correlation between stem rot severity and seedling growth parameters

Pearson's correlation analysis revealed that the lowest stem rot severity, estimated based on a necrosis index, led to significant increases in all seedling growth parameters. Indeed, the seedling height was negatively correlated to necrosis index (r = -0.471; n=190; P = 0.000). Moreover, the stem diameter was negatively linked to stem rot severity (r = -0.431; n=190; P = 0.000). Furthermore, the aerial part dry weight (r = -0.392; n=190; P = 0.000) and the root dry weight (r = -0.268; n=190; P = 0.001) were negatively correlated to the necrosis index.

#### 3.5 Growth-promoting ability of compostassociated actinomycetes on pathogen-free tomato seedlings

ANOVA analysis revealed that all growth parameters (height, stem diameter, aerial part dry weight, and root dry weight), varied significantly (at  $P \le 0.05$ ) depending on tested actinobacterial treatments. As presented in Table 3, the A4-4 isolate exhibited the highest growthpromoting ability on tomato pathogenfree seedlings where the height was enhanced by 27% over the untreated control. This parameter was also significantly increased by 15.8 and 12.1% over control following seedling treatment with A3-3and A3-4 isolates, respectively. However, no positive effect was noted based on the stem diameter. Treatment of tomato seedlings with A3-3, A4-3, A1-4, and A4-4 isolates resulted in a significant increment of the aerial part and root dry weights by 20.1-56.5% and 42.3-51%, respectively, over control. A3-3 and A5-4 isolates exhibited the highest growth-promoting potential based on the aerial part dry and the root dry weight, respectively (Table 3).

Table 3: Growth-promoting potential of compost-associated actinomycete isolates noted 35 days post-sowing on pathogen-free tomato cv. Rio Grande seedlings as compared to the untreated control.

		<b>e</b> .		
Biological treatments	Plant height (mm)	Stem diameter (mm)	Aerial part dry weight (mg)	Root dry weight (mg)
UC	81.5 ± 1.35 def	$2.28 \pm 0.10 \text{ ab}$	189.87 ± 7.10 de	$26 \pm 1.13$ bcdef
A1-3	$69.37 \pm 2.54$ g	$1.86 \pm 0.06 \text{ bc}$	$143.5 \pm 11.10 \text{ f}$	$19.37 \pm 0.82 \ def$
A2-3	$81.37 \pm 3.74 \text{ def}$	$1.81 \pm 0.01 \text{ bc}$	$142.75 \pm 1.38 \text{ f}$	$18 \pm 0.27 \text{ ef}$
A3-3	$94.37 \pm 2.56  b$	$2.43 \pm 0.16$ a	297.12 ± 23.67 a	39 ± 5.63 a
A4-3	$90.25 \pm 4.66$ bcd	$2.29 \pm 0.12 \text{ ab}$	$256.75 \pm 16.49 \text{ b}$	37 ± 5.73 a
A5-3	83.37 ± 2.09 cde	$2.02 \pm 0.16$ abc	$225.25 \pm 28.20$ bcd	$36 \pm 4.46$ ab
A8-3	83.12 ± 3.33 cde	$1.99 \pm 0.14$ abc	188.75 ± 3.25 de	29.5 ± 1.22 abcd
A9-3	$81.75 \pm 4.07 \text{ def}$	$1.67 \pm 0.08 \text{ c}$	$134.62 \pm 7.03 \text{ f}$	$18.375 \pm 1.03 \text{ def}$
A10-3	$80.62 \pm 1.64$ ef	$2.04 \pm 0.06$ abc	$210.12 \pm 7.72$ cde	31.25 ± 2.62 abc
A1-4	87.12 ± 1.76 bcde	$1.79 \pm 0.1 \text{ bc}$	233.12 ± 10.99 bc	37.25 ± 4.25 a
A2-4	83.75 ± 2.25 cde	$1.71 \pm 0.06 \text{ c}$	184.5 ± 4.32 e	$25.75 \pm 1.37$ bcdef
A3-4	91.37 ± 1.37 bc	$2.30 \pm 0.08 \text{ ab}$	186.12 ± 7.75 e	$28.75 \pm 3.62$ abcde
A4-4	103.5 ± 9.67 a	$1.99 \pm 0.11$ abc	$228.12 \pm 16.32$ bc	39.25 ± 5.99 a
A5-4	$83.5 \pm 2.46$ cde	$1.87 \pm 0.16$ bc	$219.5 \pm 8.87$ cde	39.25 ± 6.12 a
A6-4	84.87 ± 2.56 cde	$1.98 \pm 0.04$ abc	199.5 ± 2.84 cde	$33.5 \pm 2.08$ abc
A7-4	$55.12 \pm 1.53$ h	1.75 ± 0.19 c	145.25 ± 4.63 f	$24.12 \pm 1.8 \text{ cdef}$
A8-4	$69 \pm 2.07 \text{ g}$	$1.8 \pm 0.03$ bc	148.87 ± 3.39 f	24 ± 1.99 cdef
A10-4	$73.5 \pm 2.16 \text{ fg}$	$1.93 \pm 0.11$ bc	141.37 ± 5.25 f	$16.87 \pm 2.02 \text{ f}$

UC: Uninoculated and untreated control. Results are presented as means  $\pm$  SE (n = 10,  $P \le 0.05$ ). For each column, values followed by the same letter are not significantly different according to Duncan's Multiple Range test (at  $P \le 0.05$ ).

#### 4. Discussion

Several investigations have reported that microorganisms isolated from composts could enhance the growth of various crops and controlling several phytopathogens (Coelho et al., 2020; Joshi et al., 2009). In previous work, we demonstrated the capacity of composts and compost teas to suppress stem rot disease caused by *S. rolfsii* and to promote tomato growth (Ayed et al., 2018b; 2018c). Therefore, this study was more focused on the isolation and screening of actinomycetes naturally associated with 2 composts as biocontrol and growth-promoting agents. A total of 17 actinomycetes isolates were recovered from 2 selected composts C<sub>3</sub> and C<sub>4</sub>. When assessed for their ability to suppress stem rot disease, 12 actinobacterial isolates (namely A2-3, A3-3, A4-3, A5-3, A8-3, A9-3, A1-4, A2-4, A3-4, A4-4, A6-4, and A10-4) had successfully decreased disease severity on S. rolfsiiinoculated tomato cv. Rio Grande seedlings. The noted antifungal activity of actinomycetes isolates confirmed the results obtained by Anusree and Bhai (2017) who demonstrated their ability to protect pepper by 98.10% from Sclerotium foot rot disease. Also, Errakhi et al. (2007) reported the antagonistic potential of actinobacterial isolates against S. rolfsii sugar Several infecting beet. other investigations indicated that actinomycetes are promising microbial agents for the biological control of numerous fungal and bacterial plant pathogens (Dandan et al., 2018; Mingma et al., 2014; Golinska & Dahm, 2013; Kobayashi et al., 2012; Patil et al., 2010). Previous studies have also shown that actinomycetes can produce plant growth promoters (Srivastava et al., 2015; Gopalakrishnan et al., 2014). In the current study, some compost-associated actinomycetes were screened for their ability to promote the growth of tomato seedlings. Interestingly, our results demonstrated that A3-3, A4-3, A2-4, A3-4, and A4-4 isolates exhibited the highest growth-promoting potentials on S. rolfsii-inoculated tomato seedlings and that A3-3 and A4-4 were also the most effective on pathogen-free seedlings. Nevertheless, A1-3, A7-4, A8-4, and A10-4 isolates did not exhibit any positive effect on seedling growth. Thus, actinomycetes associated with composts can be explored as potential agents for seedling growthstimulation and stem rot disease biocontrol. These effects may be explained by the ability of plant root exudates to stimulate the growth

of actinomycetes and the last ones use these exudates for the synthesis of antimicrobial substances active against plant pathogens (Yuan & Crawford, 1995). Most actinomycetes belong to the genus Streptomyces and are well known as the main sources of bioactive secondary metabolites (Khanna et al., 2011; Goodfellow & Simpson 1987). Therefore, different aspects of these microorganisms have been studied, such as the production of metabolites that control plant disease and/or improve plant growth. To inhibit phytopathogens, the microbial biocontrol agents use different mechanisms such as the production of cell wall-degrading enzymes, parasitism, antibiosis, and the induction of host resistance (Palaniyandi et al., 2013). In this regard, previous findings showed the ability of actinomycetes to synthesize hydrolytic enzymes including chitinases, proteases, amylases, lipases, cellulases, or  $\beta$ -1,3 glucanase (Anusree & Bhai, 2017; Jayamurthy et al., 2014). In the same sense, actinomycetes are described as natural antibiotic producers and can inhibit various soilborne pathogens such as Fusarium oxysporum f. sp. cubense (Getha & Vikineswary, 2002), Verticillium dahliae (Aouar et al., 2012), and Rhizoctonia solani (Patil et al., 2010). Regarding plant growth efficiency promotion, the of various microorganisms, including actinomycetes, were widely reported (Himaman et al., 2016; Gopalakrishnan et al., 2014; Salla et al., 2014; Peralta et al., 2012). These agents may promote their host growth either directly by the synthesis of phytohormones (Goudjal et al., 2013), nitrogen fixation (Bibha et al., 2017), siderophores, phosphate and zinc solubilization, indole acetic acid, extracellular enzyme lipase (Anusree & Bhai, 2017); or indirectly through the suppression of their associated plant pathogens (Jose & Jha, 2016).

#### 5. Conclusion

Screened composts were found to be a potentially important source for the isolation of potent actinomycetes, acting both as biocontrol agents and as biofertilizers. Nevertheless, more investigations are needed to more understand the mechanisms of action, to identify secondary metabolites involved in the growth promotion and the stem rot biocontrol, and to identify the potent isolates; which can be used for the development of eco-friendly and economically feasible biofertilizers and bio-fungicides.

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