OPEN ACCESS International Journal of Applied Biology

International Journal of Applied Biology is licensed under a Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ISSN : 2580-2410
eISSN : 2580-2119

Studying on Antifungal Activities of *Streptomyces* Isolated from Soil and Its Biocontrol Potential Against *Fusarium* of Chili's Root Rot Disease

Nguyen Anh Dung [1*]

[1] Thu Dau Mot University, Binh Dương, Viet Nam

Abstract

Seven *Streptomyces* strains from chili farm soil, including Strep-1, Strep-2, Strep-4, Strep-5, Strep-6, Strep-8, and Strep-10, were screened for their antifungal activities against *Fusarium* sp. of chili's root rot disease. The results showed that *Streptomyces* sp. Strep-4 and *Streptomyces* sp. Strep-8 performed potential abilities to control the pathogenic *Fusarium* of which means of antifungal efficacies were 43,88 ± 3,21% and 51,8 ± 2,54%, in turn. Extensive researches on the effects of inoculum factors on antifungal activity of *Streptomyces* sp. Strep-8 resulted that this strain spontaneously synthesized the antifungal compounds to control *Fusarium* in the culture condition with pH 7, 40oC of culture temperature, and 7 days of culture time. The results proved that *Streptomyces* sp. Strep-8 with its thermophilic ability not only provided significant inhibitory activity against pathogenic *Fusarium* sp. in vitro also promised to be a potential biological control agent to eliminate this fungus on fields. Moreover, using *Streptomyces* sp. Strep-8 as a biological control agent helps reduce the use of fungicides which contributes to boosting the sustainability of agricultural ecosystems worldwide.

Article History
Received March 28, 2024
Accepted June 24, 2024

Keyword
Antifungal activity; Streptomyces; biocontrol agent; pathogenic Fusarium

Introduction

Fusarium spp. have been observed worldwide from tropical to temperate regions and even in harsh climates (Early, 2009). This genus is imperfect fungi consisting of various plant pathogenic agents (Suga & Hyakumachi, 2004). *Fusarium* species have a very broad host range resulting in causing economic losses in all cereal crops in many regions over the world (Voigt, 2002). *Fusarium* spp. have been known to be responsible to diseases of wheat, corn, barley, rice and other small grains (Osrborn & Stein, 2007). Actually, the worldwide harvested crops are recorded to be contaminated with mycotoxins from 25 to 50 percent (Ricciardi et al., 2013). Moreover, *Fusarium* mycotoxins, including fumonisins and trichothecenes produced by *Fusarium* spp. can be fatal for animals and humans (Rheeder et al., 2002), exhibit both acute and chronic toxic effects in humans and animals (Marin et al., 2013). These different toxins cause virulence during the development of diseases in plants, contaminating grains before being consumed by humans and livestock (Bakker et al., 2018). Many papers have been documented that *Fusarium* species cause a wide spectrum of infections in humans such as onychomycosis, skin infections, keratitis (Van Diepeningen et al., 2014) and involving the

skin, brain, bloodstream, lungs, eyes, and bones(Van Diepeningen et al., 2014; Garcia et al., 2015; Douglas et al., 2016).

Streptomyces is a genus of actinobacteria, inluding filamentous Gram positive soil bacteria with a high G + C content and well known for their ability as biological control agent (El-Tarabily, 2008; Caraveo et al., 2014; Soltani et al., 2015). They can produce enormous varieties of secondary metabolites that have been developed into biofungicides. (Rashad et al., 2015). The antagonistic activity of *Streptomyces* against fungal pathogens is usually related to the production of antifungal compounds (Getha et al., 2002; Fguira et al., 2005) and extracellular hydrolytic enzymes such as Chitinase and β-1,3-glucanase are considered to be important hydrolytic enzymes in the lysis of fungal cell walls (Trejo-Estrada et al., 1998; Mukherjee & Sen, 2006).

In this study, seven strains of *Streptomyces* spp. obtained from soil of chili farm in Binh Duong, Viet Nam were screened for the ability to control the *Fusarium* sp. causing the root rot disease in Chili. Besides, the antifungal activity of *Streptomyces* was also evaluated in some different inoculum conditions.

Materials and Methods

Preparation of *Fusarium* sp. and *Streptomyces* spp.

Fusarium sp. and seven strains of *Streptomyces* spp. were kindly provided by the Labolatory of Microorganism and Biochemistry, The institute of applied technology, Thu Dau Mot university, Binh Duong, Viet Nam. *Fusarium* sp. was isolated from the rotted root of chili and store on the PDA (Potato – Dextrose – Agar) slants at 5°C. The pathogenic *Fusarium* was cultivated on PDA petri disk before using in experiments. Besides, *Streptomyces* spp. were isolated from soil of chili farms in Binh Duong, Viet Nam and stored at 5°C on Gause II slants for actinobacteria. All *Streptomyces* spp. were also activated on petri disks poured into by 15 ml of Gause I agar medium before studies (Caraveo et al, 2014, Getha & Vikineswary, 2002).

Preparation of *Streptomyces* spp. antifungal supernatant (AS)

Streptomyces spp. were cultured on Gause I agar medium at 37°C for 7 days before tranfering aseptically into erlen containing 200 ml of Gause I broth. The inoculated erlens with different were continuously cultured in orbital incubator shaker at 180 rpm, 37°C for 5 days, then their biomasses were centrifuged at 4500 rpm to remove pellet and collect the supernatant for study on *Streptromyces* antifungal activity (AA) (Amini *et al.* 2016).

In vitro Bioassay of *Streptromyces* antifungal activity against *Fusarium*

Antifungal activities (AAs) of *Streptomyces* spp. against *Fusarium* sp. were determined by the agar well diffusion method.The principle was based on the diffusion of the antifungal compounds of the supernatant into the agar to inhibit the growth of *Fusarium* colony and formed inhibiting zones. Practically, using sterile cork borer to dig four wells with d = 8 mm on each Petri dish containing 15 mL of PDA medium (Potato Dextrose Agar) on four sites at equal distances from the center of plates, before dropping 100 µL of prepared *Streptomyces* supernatant into each well. Then a single eight-millimeter diameter disc of *Fusarium* sp., obtained from the already inoculated PDA plates with tested fungi (100 µL; 10^{5} spores/mL) for 5 days, was put in the middle of tested Petri dish. The same actions were carried out for the control, although sterilized distilled water was used to replace to *Streptomyces* supernatant. The experimental and control groups were were kept at 4°C for 30 mins to well diffuse the antifungal compounds. Then the PDA plates were incubated at 30°C and measured

the diameter of *Fusarium* colony in millimeter (Bauer *et al*., 1966; NCCLS, 2000; Balouirin *et al*., 2016). The experimental and control groups were replicated 3 times to calculate their means.

The modified Abbott's formula was applied to determine the antifungal efficacy (AE) of *Streptomyces* spp. supernatant against *Fuasarium* sp (Balouirin *et al*., 2016).

$$H = \frac{\mathrm{D} - \mathrm{d}}{\mathrm{D}} \text{ x } 100 \text{ (percent control \%)}$$

Note:

D : the mean of diameter of *Fusarium* colony (MDFC) in control groups (mm)

d : the mean of diameter of *Fusatium* colony in experimental groups (mm)

H : the antifungal activity efficacy of *Streptomyces* supernatant (%)

Screening antifungal activity of *Streptomyces* supernatants in different initial pH scales

Streptomyces spp. were cultured on Gause I agar medium at 37°C for 7 days before tranfering aseptically into five erlens containing 200 ml of Gause I broth with the initial pH scales 4, 5, 6, 7, 8. The pH scale of medium was measured by pH meter (HANNA) and adjusted by HCl 0,1N and NaOH 0,1N. The inoculated erlens with different initial pH scales were continuously cultured in orbital incubator shaker at 180 rpm, 37°C for 5 days, then the *Streptomyces* biomass was centrifuged at 4500 rpm to remove pellet and collect their supernatant for testing the AA against *Fusarium* sp. by the agar well diffusion method. The optimistic initial pH level induced the highest AE was recorded and applied for next experiments.

Screening antifungal activity of *Streptomyces* supernatants in different conditions of culturing temperature

Streptomyces spp. were cultured on Gause I agar medium at 37°C for 7 days before tranfering aseptically into five erlens containing 200 ml of Gause I broth with the recorded optimistic initial pH. The pH scale of medium was measured by pH meter and adjusted by HCl 0,1N and NaOH 0,1N. The inoculated erlens were continuously cultured in orbital incubator shaker at 180 rpm and different culturing temperature including 25°C, 30°C, 35°C, 40°C and 45°C for 5 days, then the *Streptomyces* biomass was centrifuged at 4500 rpm to remove pellet and collect the supernatant for testing the AA against *Fusarium* sp. by the agar well diffusion method. The optimistic temperature to induced the highest AE was recorded and applied for next experiments.

Screening antifungal activity of *Streptomyces* supernatants at different culture time intervals

Streptomyces spp. were cultured on Gause I agar medium at 37°C for 7 days before tranfering aseptically into five erlens containing 200 ml of Gause I broth with the recorded optimistic initial pH. The pH scale of medium was measured by pH meter and adjusted by HCl 0,1N and NaOH 0,1N. The inoculated erlens were continuously cultured in orbital incubator shaker at 180 rpm and optimistic culturing temperature for different time intervals including 3 days, 5 days, 7 days, 9 days and 11 days. Then the *Streptomyces* biomass was centrifuged at 4500 rpm to remove pellet and collect the supernatant for testing the AA against *Fusarium* by the agar well diffusion method. The optimistic time interval for the highest AE was recorded and applied for next experiments.

Data Analysis

The information recorded in the groups under studies and controls was analysed by t-Test for comparing means using STATGRAPHICS CENTURION XIX licensed software. Duncan's Multiple Range Test was utilized to compare means. ($p < 0.05$).

Results and Discussion

Antifungal activities of *Streptomyces* spp. against *Fusarium* sp.

Seven strains of *Streptomyces* were obtained from soil and screened for their antifungal activities (AAs) against *Fusarium* sp. to isolate the strongest strain in term of *Fusarium* sp. control. After culturing and collecting, their supernatants were examined for AAs by the agar well diffusion method on the Petri dishes containing PGA medium. The results of the screening experiments showed that most of *Streptomyces* strains were able to control *Fusarium* sp. (Table 1). Means of antifungal efficacies (AEs) were recorded differentially among screened *Streptomyces* strains. Among them, Strep-1, Strep-2, Strep-5, Strep-6 and Strep-10 performed very low AEs in term of *Fusarium* control of which were 6,61 ± 1,25%, 3,64 ± 2,18%, 1,96 ± 0,23%, 3,86 ± 1,41% and 5,62 ± 1,01%, respectively. On the contrary, Strep-4 and Strep-8 showed their potential abilities to control the pathogenic *Fusarium* of which means of AEs were 43,88 ± 3,21% and 51,8 ± 2,54%, in turn, much higher than the others ($p<0.05$). The potential abilities to control *Fusarium* sp. of Strep-4 and Strep-8 were easily observed on the tested Petri dishes by their supernatants in which the means of *Fusarium* colony diameters (FuCDs) were very low growth including 26,16 ± 2,25 mm of Strep-4 and 22,16 ± 3,68 mm of Strep-8 compared to the 50,5 ± 1,32 mm of the distilled-water control group. Moreover, the pathogenic *Fusarium* mycelia seemed to be inhibited to grow nearby the wells on the tested dishes whereas the mean of FuCD was 50,5 ± 1,32 mm and strongly overgrew the wells containing distilled water on control tested dishes (Figure 2). In comparison between the examined group, obviously, the Strep-8 showed its domination in term of *Fusarium* sp. control (the smallest mean of FuCD and the highest mean of AE) (Figure 1) resulting in the Strep-8 was the chosen strain for deeper experiments on AA against *Fusarium* sp. of chili's rotted root disease.

Table 1. Antifungal activity of *Streptomyces* spp. against *Fusarium* sp.

Strains	d (Mean ± Sd, mm)	D (Mean ± Sd, mm)	D – d (Mean ± Sd, mm)	Efficacy (Mean ± Sd, %)
Strep-1	47,16 ± 5,00 ^{c}	50,5 ± 1,32	3.34 ± 1.21^{a}	6,61 ± 1,25^{a}
Strep-2	48,66 ± 6,33 ^{c}		1.84 ± 0.25^{a}	3,64 ± 2,18^{a}
Strep-4	26,16 ± 2,25 ^{b}		24.34 ± 2.31^{c}	43,88 ± 3,21^{c}
Strep-5	49,51 ± 2,78^{c}		0.99 ± 0.11^{a}	1,96 ± 0,23^{a}
Strep-6	48,55 ± 3,5^{c}		1.95 ± 0.75^{a}	3,86 ± 1,41^{a}
Strep-8	22,16 ± 3,68 ^{a}		28.34 ± 2.15^{d}	51,8 ± 2,54^{d}
Strep-10	47,66 ± 4,93^{c}		2.84 ± 1.22^{a}	5,62 ± 1,01^{a}

Note: Means with the same letters in the same column are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$)

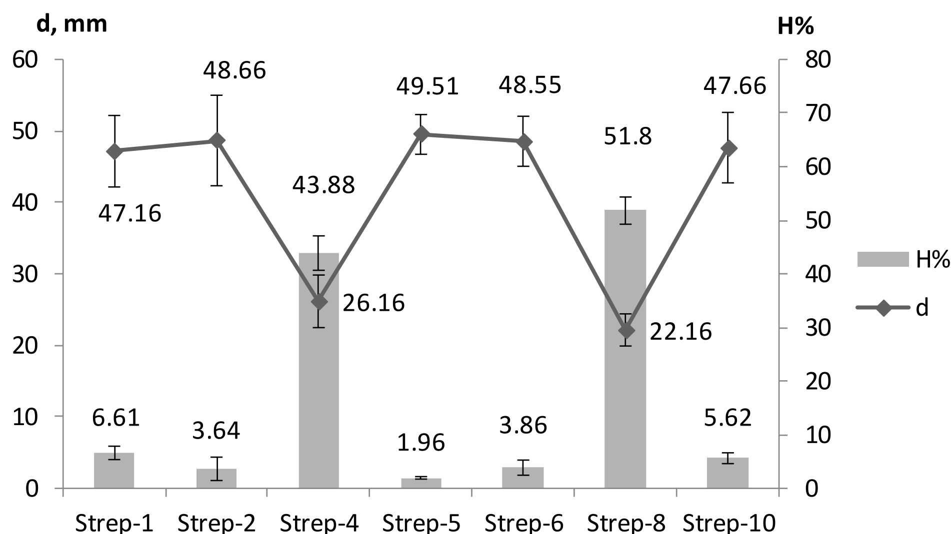


Figure 1. The graph showed means of FuCDs and AEs of seven *Streptomyces* strains (p<0.05). The smaller mean of FuCD, the better AE of *Streptomyces*.

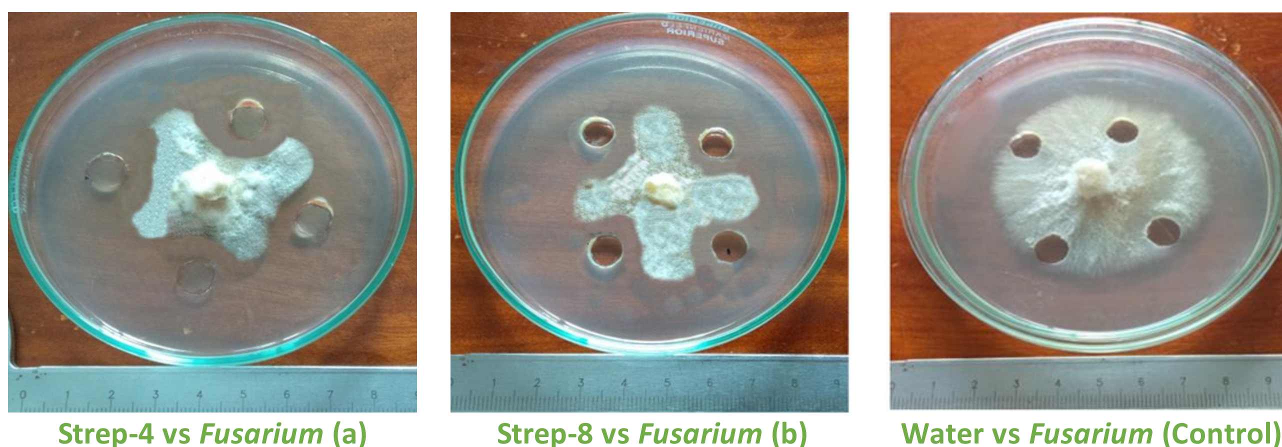


Figure 2. *Fusarium* mycelia on the PGA Petri tested dishes with wells containing supernatants and distilled water.
a - Strep-4 supernatant; b – Strep-8 supernatant; Control – distilled water.

The effect of initial pH levels on antifungal activities against *Fusarium* of *Streptomyces* sp. Strep-8

pH is one of inoculation factors not only affects on microbial growth, including *Streptomyces* spp. are not exceptional, also the ability to produce antifungal compounds. This test was designed to investigate and identified the optimal pH level for AA of Strep-8 against *Fusarium* sp.. The experimental results showed the pH played a crucial role in controlling the pathogenic *Fusarium* of Strep-8 (Table 2). The means of FuCDs in the acidic pH 4 and pH 5 tested groups were 46.53 ± 1.50 mm and 41.33 ± 2.08 mm, respectively, whereas the mean of FuCD in control group was 50.67 ± 1.53 mm. As a result, means of AEs of acidic pH 4 and pH 5 were also low including 8.16 ± 2.96% and 18.43 ± 4.11%, in turn. The same result was observed in the pH 8 tested group with 47.5 ± 1.33 mm for the mean of FuCD and 6.26 ± 2.61% for the antifungal efficacy. On the other hand, the smaller means of FuCDs and

the higher means of AEs of pH 6 and pH 7 experimental groups were differently recorded from the others (P<0.05). The treatment group with the pH 6 showed the mean of FuCD at 25.1 ± 1.45 mm and the mean of AE at 50.46 ± 2.87% whilst in the neutral pH 7 tested group, these indexes were recorded the smallest one at 17.12 ± 1.26 mm and the highest one at 66.12 ± 2.48% between tested groups. On the experimental agar Petri dishes, likely to the pathogenic fungi of control group with distilled water in wells, the *Fusarium* mycelia of pH 4, pH 5, and pH 8 tested groups grew strongly even to pass through the wells containing Strep-8's culturing supernatant. In contrast, in the pH 6 and pH 7 tested dishes, the added supernatants inhibited the growth of *Fusarium* mycelia near to the wells (Figure 3). Actually, the pH factor strongly affected on Strep-8 to produce antifungal compounds. The acidic pH (pH 4 and pH 5) and the basic pH (pH 8) did not boost Strep-8 to spontaneously synthesize the antifungal compounds to control *Fusarium*, which were forcefully enhanced by neutral pH 7.

Table 2. Antifungal activities of *Streptomyces* sp. (Strep-8) against *Fusarium* sp. under the effect of different initial pH levels

pH	d (Mean ± sd, mm)	D (Mean ± sd, mm)	D – d (Mean ± sd, mm)	H (Mean ± sd, %)
4	46.53 ± 1.50^{d}	50.67 ± 1.53	4.14 ± 1.50^{a}	8.16 ± 2.96^{a}
5	41.33 ± 2.08^{c}		9.34 ± 2.08^{b}	18.43 ± 4.11^{b}
6	25.1 ± 1.45^{b}		25.57 ± 1.45^{c}	50.46 ± 2.87^{c}
7	17.12 ± 1.26^{a}		33.50 ± 1.26^{d}	66.12 ± 2.48^{d}
8	47.5 ± 1.33^{d}		3.17 ± 1.32^{a}	6.26 ± 2.61^{a}

Note: Means with the same letters in the same column are not significantly different according to Duncan's Multiple Range Test (P < 0.05)

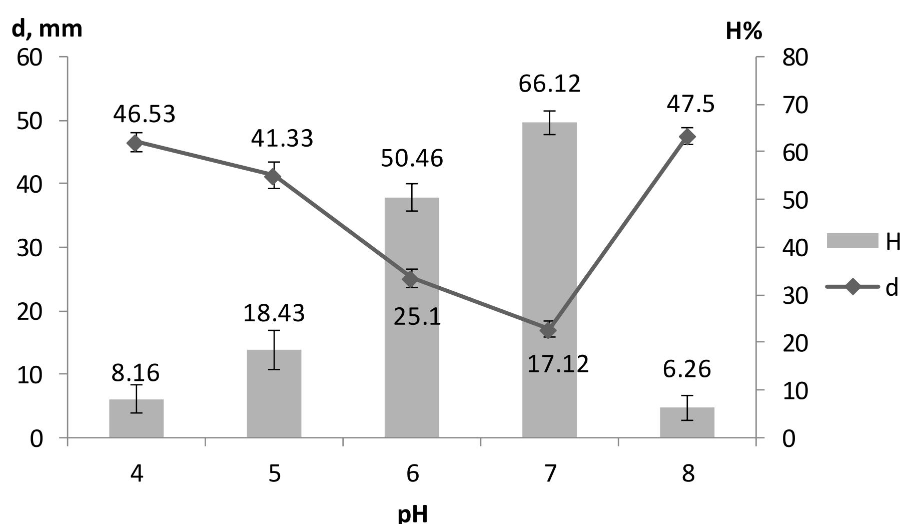


Figure 3. The graph showed the means of FuCDs and AEs of Strep-8 under different levels of pH (p<0.05). The smaller mean of FuCD, the better AE of *Streptomyces*.

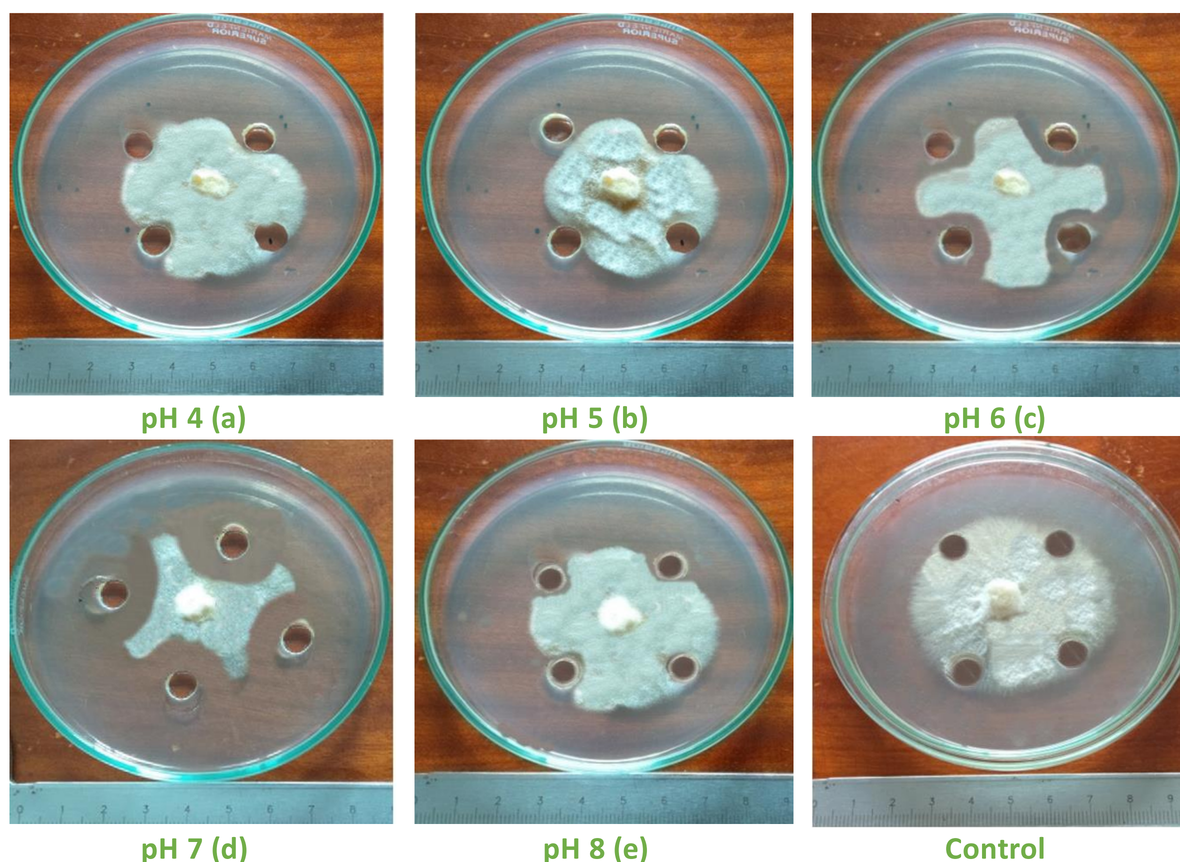


Figure 4. *Fusarium* mycelia on the Petri dishes with wells containing supernatants collected from Strep-8 biomasses at different initial pH levels for tested group and distilled water for control group. a,b,c,d,e - Strep-8 supernatants from different pH levels including 4, 5, 6, 7 and 8, respectively; Control - distilled water.

The effect of culture temperature on antifungal activity against *Fusarium* of *Streptomyces* sp. Strep-8

To find out the culture temperature (CT) affected on the antifungal activity of Strep-8 against *Fusarium*. An experiment was carried out by culturing Strep-8 at different CT conditions including 25°C, 30°C, 35°C, 40°C and 45°C with the optimistic pH 7 from the above experiment, the results were demonstrated on table 3. Obviously, under the effects of culture temperatures (CTs), three groups belong to different rates of AAs were performed by Strep-8. On Petri dishes, the supernatants were obtained from Strep-8 biomasses at 25°C and 30°C showed their weak AAs against *Fusarium* of which means of FuCDs were largely sized at 32.17 ± 2.75 mm and 30.27 ± 1.42 mm resulting in low means of AEs at 37.33 ± 5.37% and 41.03 ± 2.76%, respectively. The CT at 35°C initially boosted Strep-8 to dramatically improve the antifungal activity with the mean of *Fu*CD on the tested dishes at 18.17 ± 1.26 mm and 64.61 ± 2.45% for the mean of AE, showed the smallest mean of *Fu*CD on the tested dishes at 11.83 ± 0.76 mm and the highest mean of AE at 76.95 ±1.49% in the 40°C experiment before slighly increased to 16.5 ± 1.5 mm of *Fu*CD and reduced to 67.86 ± 2.93% of AE in the 45°C experiment. Moreover, obviously, the Petri tested dishes showed that CT strongly affected on the antagonistic activity of Strep-8 against *Fusarium*, of which the obseverved pathogenic *Fusarium* colonies were bordered by the antifungal compounds in wells excepted the control experiment with distilled water (figure 6). As a result, 40°C was considered as the optimistic CT for Strep-8 to produce antifungal compounds.

Table 3. Antifungal activities of *Streptomyces* sp. (Strep-8) against *Fusarium* sp. under the effect of different levels of culture temperature

Temp °C	d (Mean ± Sd, mm)	D (Mean ± Sd, mm)	D – d (Mean ± Sd, mm)	H (Mean ± Sd, %)
25	32.17 ± 2.75^{c}		19.16 ± 2.75^{a}	37.33 ± 5.37^{a}
30	30.27 ± 1.42^{c}		21.06 ± 1.42^{a}	41.03±2.76^{a}
35	18.17 ± 1.26^{b}	51.33 ± 1.53	33.16 ± 1.26^{b}	64.61 ± 2.45^{b}
40	**11.83 ± 0.76^{a}**		**39.50 ± 0.76^{c}**	**76.95 ±1.49^{c}**
45	16.5 ± 1.5^{b}		34.83 ±1.5^{b}	67.86 ± 2.93^{b}

Note: Means with the same letters in the same column are not significantly different according to Duncan's Multiple Range Test (P < 0.05)

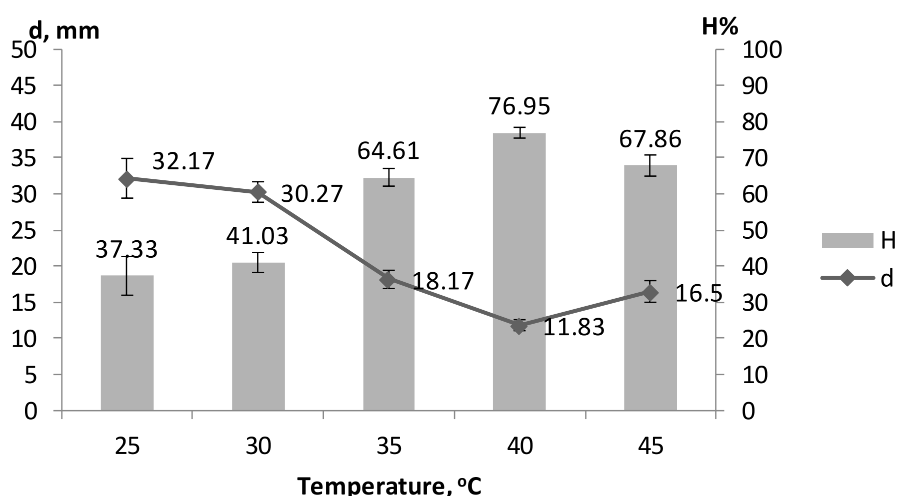
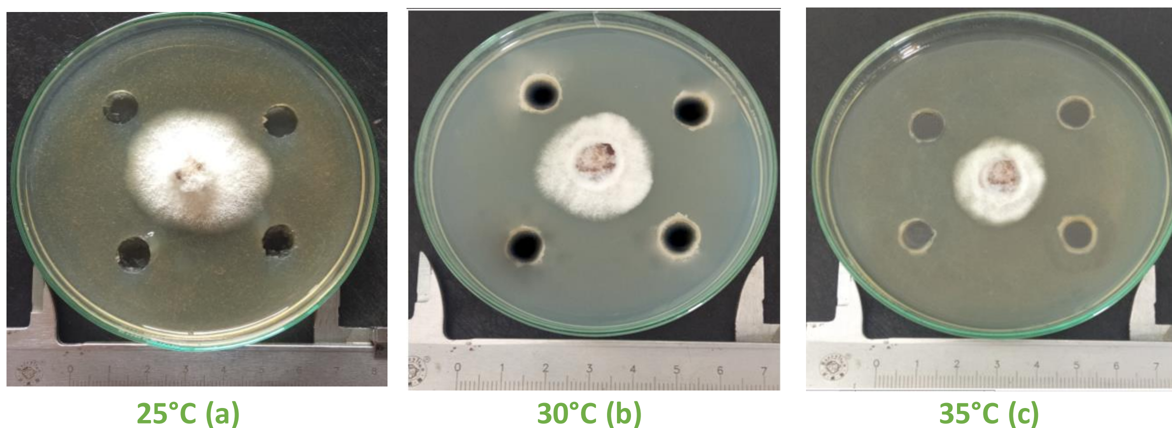


Figure 5. The graph showed means of FuCDs and AEs of Strep-8 under different levels of CT (p<0.05). The smaller mean of FuCD, the better AE of *Streptomyces*



25°C (a) **30°C (b)** **35°C (c)**

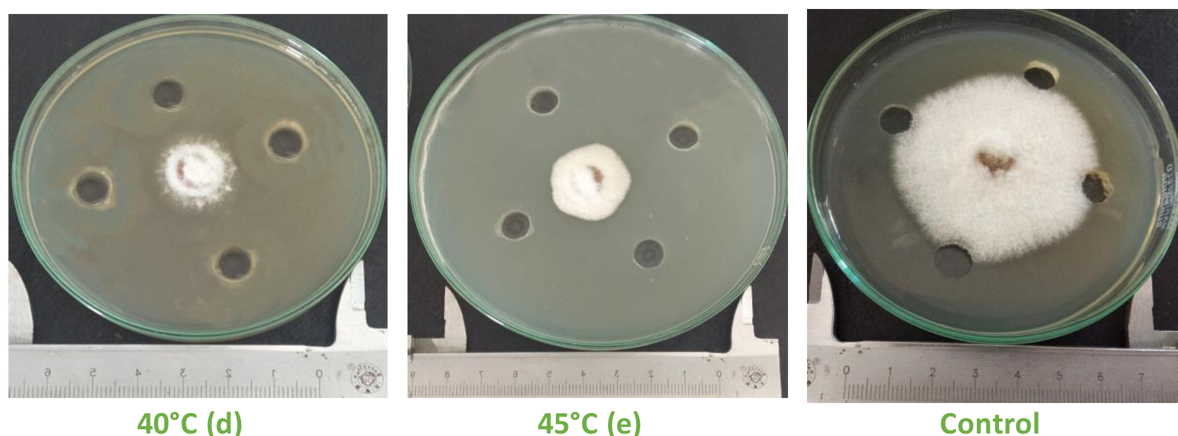


Figure 6. ***Fusarium*** **mycelia on the Petri dishes with wells containing supernatants collected from Strep-8 biomasses at different CT levels for tested group and distilled water for control group. a,b,c,d,e - Strep-8 supernatants at different CT levels including 25°C, 30°C, 35°C, 40°C and 45°C, respectively; Control - distilled water**

The effect of culture time on antifungal activity against *Fusarium* of *Streptomyces* sp. Strep-8

To find out the culture time (CTi) affected on the AA of Strep-8 against *Fusarium*. Strep-8 was cultured at different periods of time including 3 days, 5 days, 7 days, 9 days and 11 days at the optimistic pH 7 and 40°C from the above experiments of which the results were demonstrated on table 4. Strep-8 showed strong AA against *Fusarium* soon after three days of CTi of which the mean FuCD was measured at 18.67 ± 1.53 mm resulting in the high mean of AE at 64.10 ± 2.94% which dramatically decreased to 12.33 ± 0.58 mm and increased to 76.28 ± 1.1% after 5 days of CTi, in turn. Continuously, at the 7th day of the culture process, the smallest mean of FuCD and highest mean of AE were recorded at 9.93 ± 0.12 mm and 80.90 ± 0.22%. However, the AA of Strep-8 initially showed the reduction when the means of FuCD and of AE were 11.83 ± 1.44 mm and 77.25 ± 2.78%, which were 16.33 ± 1.04 mm and 68.59 ± 1.99% of the experiments of 9 days and 11 days, respectively. In the Petri tested dishes proved CTi strongly affected on the AA of Strep-8 against *Fusarium*, of which the obseverved pathogenic fungus colonies were bordered by the antifungal compounds in wells excepted the control experiment with distilled water (figure 8). As a result, the optimistic CTi for Strep-8 to produce antifungal compounds was 7 days.

Table 4. Antifungal activities of *Streptomyces* sp. (Strep-8) against *Fusarium* sp. under different periods of culture time

Time (days)	d (Mean ± Sd, mm)	D (Mean ± Sd, mm)	D – d (Mean ± Sd, mm)	H (Mean ± Sd, %)
3	18.67 ± 1.53^{d}		33.33 ± 1.53^{a}	64.10 ± 2.94^{a}
5	12.33 ± 0.58^{b}		39.67 ± 0.58^{c}	76.28 ± 1.11^{c}
7	9.93 ± 0.12^{a}	52 ± 1.73	42.07 ± 0.12^{d}	80.90 ± 0.22^{d}
9	11.83 ± 1.44^{b}		40.17 ± 1.44^{cd}	77.25 ± 2.78^{cd}
11	16.33 ± 1.04^{c}		35.67 ± 1.04^{b}	68.59 ± 1.99^{b}

Note: Means with the same letters in the same column are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$)

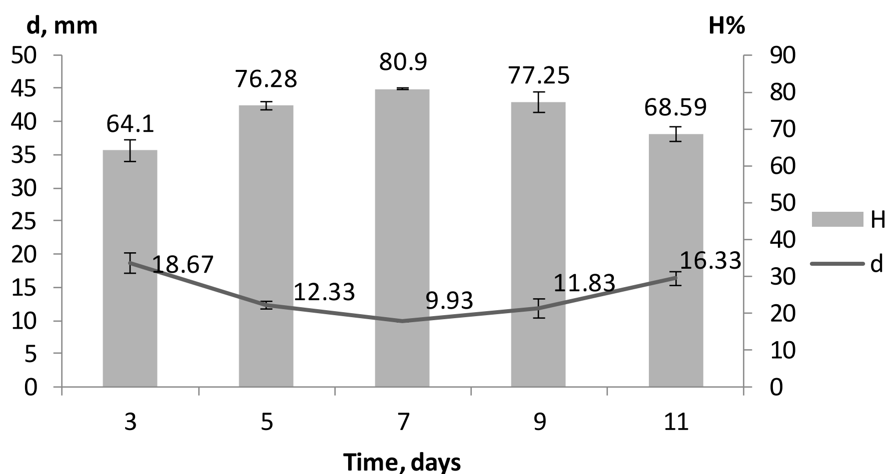


Figure 7. The graph showed means of FuCDs and AEs of Strep-8 under different periods of CT ($p<0.05$). The smaller mean of FuCD, the better AE of *Streptomyces*

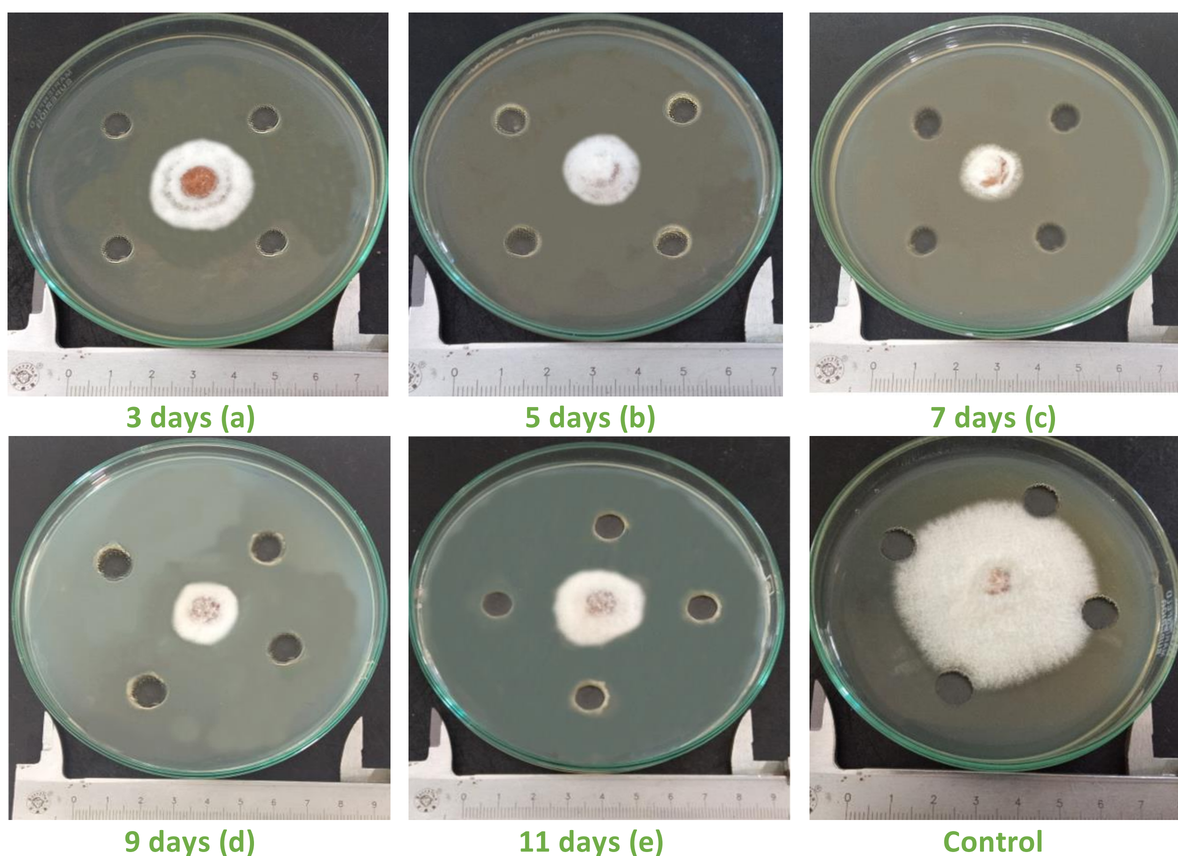


Figure 8. *Fusarium* mycelia on the Petri tested dishes with wells containing supernatants obtained from Strep-8 biomasses at different periods of CTi for tested group and distilled water for control group. a,b,c,d,e - Strep-8 supernatants at at different periods of CT including 3 days, 5 days, 7 days, 9 days and 11days, respectively and Control - distilled water.

In terms of antifungal activity, *Streptomyces* sp. YYS-7 has a broadspectrum antifungal activity against seven phytopathogenic fungi of which inhibition percentage of mycelial growth in dual culture assay were showed as follows *C. fallax* (76.07 ± 1.97), *C. musae* (51.07

± 1.57), *C. fragariae* (64.50 ± 0.7), *C. gloeosporioides* (72.75 ± 1.06), *F. oxysporum cucumerinum* (59.25 ± 1.77), *C. acutatum* (74.80 ± 0.28) and *F. graminearum* (65.26 ± 0.5). Moreover, crude extract of *Streptomyces* sp. YYS-7 inhibited the pathogenic mycelial growths which in turn were C. fallax (69.51 ± 1.35), *C. gloeosporioides* (47.95 ± 4.92), *C. fragariae* (53.21 ± 2.91), *F. oxysporum cucumerinum* (65 ± 7.85), *C. acutatum* (63.76 ± 1.65), *F. graminearum* (48.61 ± 1.28), and C. musae (53.07 ± 1.80) (Wei et al., 2020). Besides, 126 *Streptomyces* isolates were recovered from rhizosphere soils of 13 different commercial vegetable greenhouses in Iran were screened for *in vitro* antagonism against *Fusarium oxysporum* f. sp. *lycopersici* race 3 (*FOL*) of *Fusarium* tomato wilt. Among them, six isolates showed more than 30% inhibitory effect against *FOL* in dual culture test. The percentages of growth inhibition were 69%, 49%, 48%, 42%, 39%, and 38%, which were recorded for IC10, IT25, Y17, TO612, Y28, and IC13, respectively. Moreover, *in vitro* Plant growth promoting experiment on Tomato showed that strains IC10 and Y28 increased shoot length and shoot fresh and dry weight compared to not inoculated control plants, of which phenotypic characterization and 16S rRNA gene sequencing were closely related to *S. enissocaesilis* and *S. rochei*, respectively (Abbasi et al., 2019). A total 20 isolates of 229 actinomycete strains with different colony morphology obtained from the rhizosphere soil samples performed potential of antifungal activities against *Fusarium oxysporum* f. sp. cubense Tropical Race 4 (Foc TR4). Especially, the strain *Streptomyces* sp. YYS-7 had the strongest antifungal activity of which inhibition zone after treatment with the strain YYS-7 was reduced to 23.82 mm ± 0.25 compared to the growth diameter (79.18 mm ± 0.63) of Foc TR4 in the control plate. Besides, the crude extract of strain YYS-7 effectively inhibited the growth of Foc TR4 and the inhibition zone was 36.42 ± 0.35 mm. The inhibition percentages of mycelial growth were 69.91% and 57.02%, respectively (Wei et al., 2020). *Streptomyces* strains including KS31, KS55, KS58, KS62 and KS112 were isolated from chickpea rhizospheric soils showed strong inhibitory effects against chickpea *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris.* All bacterial strains inhibited mycelial growth of the pathogen ranging from 26 to 44.2% in dual culture assay. The non-volatile extract of five of the *Streptomyces* strains inhibited more than 50% growth of the pathogen, whereas volatile compounds were effective on mycelial growth inhibition ranging from 20.2% to 33.4% (Amini et al., 2016).

With regard to the effects of innoculum factors on antifungal activity of *Streptomyces* spp, in vitro experiments demonstrated that the production of antifungal metabolites in culture broth at 28°C by strain MR14 commenced on 1st day of incubation, reached the maximum after 4 days and then declined as the incubation was further extended. 4-day-old culture supernatant of *Streptomyces* sp. MR14 antagonised against a variety of fungal phytopathogens of which inhibition zones were from 31± 0.0 to 11 ± 0.5 mm, including *P. oryzae* (31 mm) *Exserohilum* sp. and *C. gloeosporioides* (29 mm), *C. acutatum* (28 mm), *A. brassicicola* (27 mm), *A. alternata* (26 mm), *A. solani* (26 mm), *A. mali* (25 mm), *C. herbarum* (21 mm), and weak activity was detected against *F. monilifome* (16 mm), *C. beticola* (16 mm) and *F. oxysporum* (11 mm) (Kaur et al., 2019). More than 100 Actinomycetes isolates were screened for their antifungal activities against *F. solani* f.sp of Black root rot. pisi the pathogen. The results of sequence analysis of 16S rDNA using NCBI BLAST method for GenBank sequence comparison showed that three most effective antagonist isolates were S3, S12 and S40 identified with *Streptomyces antibioticus* and *Streptomyces peruviensis*, respectively. Moreover, the agar well diffusion assays of the crude extracts of them commenced to perform week antifungal activities of which all inhibition zones were under 10 mm on the 4th day of the culture process. The isolate S40 showed its maximum antifungal

activity at 18 mm of inhibition zone on the 8th day culture process, while two isolates S3 and S12 reached their maximum peaks of inhibitory activites at 17 mm and 18 mm on the 11th day of the culture process, in turn. After that, their antifungal activities reduced gradually to under 10 mm again on the end 25th day of the culture process (Soltanzadeh et al., 2016). *Streptomyces* g10 strain was tested for its ability to inhibit the growth of *Fusarium oxysporum* f.sp. cubense (Foc) race 1, Foc race 2 and Foc race 4. As a result, *Streptomyces* g10 strongly peformed antifungal activity against all three tested Foc races of which inhibition percentages of mycelial growth were 80%, 88% and 82% on the second day, 87%, 93% and 88% on the fourth day, 88%, 93% and 90% on the sixth day, and 89%, 93% and 92,5% on the eighth day against Foc race 1, Foc race 2 and Foc race 4, in turn. *Streptomyces* g10 showed its highest antifungal efficacy against all Foc races after eight days of incubation (Getha et al., 2005). Among thirty-seven actinobacterial isolates were recovered from the rhizosphere of healthy sugar beet plants that were screened for their potential to antagonize *F. oxysporum* (F186) in vitro, *Streptomyces* SB3-15 and *Streptomyces* SB2-23 showed their outstanding antagonistic activities against the target pathogen of which colonies were narrow and oval compared to the negative control. In detail, the mean mycelial growth diameter of F186 and inhibitory rates were 4.41 cm and 48.24%, 3.2 cm and 62.35% after dual culture with strains SB3-15 and SB2-23 for 7 days at 28°C, respectively, compared to 8.50 cm of F186 in negative control (Abdelghany et al., 2024).

In comparison, *Streptomyces* sp. Strep-8 significantly controlled on *Fusarium* sp. of which AE was higher than 50% in the screening test on AA against *Fusarium* sp. and performed the highest at 80.90 ± 0.22% in the optimal culture condition with neutral pH, 40°C of culture temperature, and 7 days of culture time. Obviously, *Streptomyces* sp. Strep-8 outstandingly inhibited pathogenic *Fusarium* compared to other studied *Streptomyces* strains including *Streptomyces* sp. YYS-7 against *F. oxysporum cucumerinum* (65 ± 7.85%), *F. graminearum* (48.61 ± 1.28%) and Foc TR4 (69.91%) (Wei et al., 2020). *S. enissocaesilis* against *Fusarium oxysporum* f. sp. *lycopersici* race 3 (69%) (Abbasi et al., 2019), five strains KS31, KS55, KS58, KS62 and KS112 of *Streptomyces* against *Fusarium oxysporum* f. sp. *ciceris* from 26% to 44.2% (Amini et al., 2016), *Streptomyces* SB3-15 and *Streptomyces* SB2-23 against *F. oxysporum* (F186) 48.24% and 62.35%, respectively (Abdelghany et al., 2024). More interestingly, the AE of *Streptomyces* sp. Strep-8 against *Fusarium* sp. was lower than 50% when the culture temperature was lower 30°C, significantly increased to 64.61 ± 2.45% at 35°C, topped at 76.95 ±1.49% at 40°C before slightly decreased to 67.86 ± 2.93% at 45°C.

Conclusion

In conclusion, the results proved that *Streptomyces* sp. Strep-8 with its thermophilic ability not only provided significant inhibitory activity against pathogenic *Fusarium* sp. in vitro also promised to be a potential biological control agent to eliminate this fungus on fields. Moreover, using *Streptomyces* sp. Strep-8 as a biological control agent helps reduce the use of fungicides which contributes to boosting the sustainability of agricultural ecosystems worldwide.

References

Abbasi, S., Safaie, N., Sadeghi, A. & Shamsbakhsh, M. (2019). *Streptomyces* Strains Induce Resistance to *Fusarium oxysporum* f. sp. lycopersici Race 3 in Tomato Through Different Molecular Mechanisms. Front. Microbiol, 10:1505. https://doi.org/10.3389/fmicb.2019.01505

Abdelghany, W. R., Yassin, A. S., Abu-Ellail, F. F. B., Al-Khalaf A. A., Omara R. I. & Hozzein W. N. (2024). Combatting Sugar Beet Root Rot: *Streptomyces* Strains' Efficacy against *Fusarium oxysporum*. *Plants*, 13, 311.https://doi.org/10.3390/plants13020311

Amini J., Agapoor Z. & Ashengroph M. (2016). Evaluation of *Streptomyces* spp. against *Fusarium oxysporum* f. sp. ciceris for the management of chickpea wilt. *Journal of Plant Protection Research*, 56(3), 257-264. https://doi.org/10.1515/jppr-2016-0038

Bakker M. G., Brown D. W., Kelly A. C., Kim H. S., Kurtzman C. P., Mccormick S. P., O'Donnell K. L., Proctor R. H., Vaughan M. M. & Ward T. J. (2018). *Fusarium* mycotoxins: A transdisciplinary overview. *Can. J. Plant Pathol*., 2, 161-171. https://doi.org/10.1080/07060661.2018.1433720

Balouirin M.; Sadiki M. and Ibnsouda S.K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6, 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

Bauer A. W., Kirby W. M. M., Sherris J. C. & Turck M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Patho*., 45, 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493

Caraveo L., Medina H., Rodriguez-Buenfil I., Montalvo-Romero C. & Evangelista-Martinez Z. (2014). A simple plate-assay for screening extracellular naringinase produced by Streptomycetes. *J. Microbiol Methods*, 102, 8–11. https://doi.org/10.1016/j.mimet.2014.04.003

Douglas A. P., Chen S. C. A. & Slavin M. A. (2016). Emerging infections caused by non-*Aspergillus* filamentous fungi. *Clin Microbiol Infect*, 22(8), 670-80. https://doi.org/10.1016/j.cmi.2016.01.011

Early R. (2009). Pathogen control in primary production: crop foods. pp205-279. Foodborne Pathogens (Second Edition), Blackburn C de W and McClure P J. Woodhead Publishing, UK.https://doi.org/10.1533/9781845696337.1.205

El-Tarabily K. A. & Sivasithamparam K. (2006). Non-streptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. *Soil Biol Biochem*, 38, 1505–1520.https://doi.org/10.1016/j.soilbio.2005.12.017

Fguira L. F. B., Fotso S., Ameur-Mehdi R. B., Mellouli L. & Laatsch H. (2005). Purification and structure elucidation of antifungal and antibacterial activities of newly isolated *Streptomyces* sp. strain US80. *Res Microbiol*, 156, 341-7. https://doi.org/10.1016/j.resmic.2004.10.006

Garcia R. R., Min Z., Narasimhan S. & Bhanot N. (2015) *Fusarium* brain abscess: case report and literature review. *Mycoses*, 58(1), 22–6.https://doi.org/10.1111/myc.12271

Getha K. & Vikineswary S. (2002). Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f. sp. *cubense* race 4: Indirect evidence for the role of antibiosis in the antagonistic process. *J Ind Microbiol Biotechnol*, 28, 303–10. https://doi.org/10.1038/sj/jim/7000248

Getha K., Vikineswary S., Wong W. H., Seki T., Ward A. & Goodfellow M. (2005). Evaluation of *Streptomyces* sp. strain g10 for suppression of *Fusarium* wilt and rhizosphere colonization in pot-grown banana plantlets. *J Ind Microbiol Biotechnol*, 32, 24–32. https://doi.org/10.1007/s10295-004-0199-5

Kaur T., Rani R. & Manhas R. K. (2019). Biocontrol and plant growth promoting potential of phylogenetically new *Streptomyces* sp. MR14 of rhizospheric origin. *AMB Expr,* 9(1), 125.https://doi.org/10.1186/s13568-019-0849-7

Marin S., Ramos A. J., Cano-Sancho G. & Sanchis V. (2013). Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology : an international journal published for the British Industrial Biological Research Association*, 60, 218–237.https://doi.org/10.1016/j.fct.2013.07.047

Mukherjee G. & Sen S. K. (2006). Purification, Characterization, and antifungal activity of chitinase from *Streptomyces venezuelae* P10. *Curr Microbiol*, 53, 265-9.https://doi.org/10.1007/s00284-005-0412-4

National Committee for Clinical Laboratory Standards. (2000). Performance standards for antimicrobial disk susceptibility tests, 7th ed. Approved standard M2–A7. Wayne, Pa: National Committee for Clinical Laboratory Standards.

Osborne L. E. & Stein J. M. (2007). Epidemiology of *Fusarium* head blight on small-grain cereals. *International Journal of Food Microbiology*. 119, 103–108.https://doi.org/10.1016/j.ijfoodmicro.2007.07.032

Rashad F. M., Fathy H. M., El-Zayat A. S. & Elghonaimy A. M. (2015). Isolation and characterization of multifunctional *Streptomyces* species with antimicrobial, nematicidal and phytohormone activities from marine environments in Egypt. *Microbiol Res*. 175, 34–47. https://doi.org/10.1016/j.micres.2015.03.002

Rheeder J. P., Marasas W. F. & Vismer H. F. (2002). Production of fumonisin analogs by *Fusarium* species. *Appl. Environ. Microbiol*, 5, 2101-2105. https://doi.org/10.1128/AEM.68.5.2101-2105.2002

Ricciardi C., Castagna R., Ferrante I., Frascella F., Marasso S. L., Ricci A., Canavese G., Lore A., Prelle A., Gullino M. L. & Spadaro D. (2013). Development of a microcantilever-based immunosensing method for mycotoxin detection. *Biosensors & Bioelectronics*, 40, 233–239.https://doi.org/10.1016/j.bios.2012.07.029

Soltani N. M., Khatami M. & Shahidi B. H. (2015). *Streptomyces somaliensis* mediated green synthesis of silver anoparticles. *Nanomed J*, 2, 217–222. https://doi.org/10.7508/NMJ.2015.03.007

Soltanzadeh M., Soltani N. M. & Shahidi B. G. H. (2016). Application of Soilborne Actinomycetes for Biological Control against *Fusarium* Wilt of Chickpea (Cicer arietinum) caused by *Fusarium* solani fsp pisi. *Journal of Phytopathology*, 164(11-12), 967-978. https://doi.org/10.1111/jph.12517

Suga H. & Hyakumachi M. (2004). Genomics of phytopathogenic *Fusarium*. In: Applied *Mycology and Biotechnology. Elsevier*, 161-189.https://doi.org/10.1016/S1874-5334(04)80009-1

Trejo-Estrada S. R., Paszczynski A. & Crawford D. L. (1998). Antibiotics and enzymes produced by the biocontrol agent *Streptomyces violaceusniger* YCED-9. *J Ind Microbiol Biotechnol*, 21, 81-90.https://doi.org/10.1038/sj.jim.2900549

Van Diepeningen A. D., Al-Hatmi A. B. M. S., Brankovics B. & de Hoog G. S. (2014). Taxonomy and clinical spectra of *Fusarium* species: where do we stand in 2014. *Curr Clin Microbiol Rep*, 1(1-2), 10–8.https://doi.org/10.1007/s40588-014-0003-x

Van Diepeningen A. D., Feng P., Ahmed S., Sudhadham M., Bunyaratavej S. & de Hoog G. S. (2015). Spectrum of *Fusarium* infections in tropical dermatology evidenced by multilocus sequencing typing diagnostics. *Mycoses*, 58(1), 48–57.https://doi.org/10.1111/myc.12273

Voigt K. (2002). Management of *Fusarium* Diseases. pp217-242. Agricultural applications. Kempken F. Springer.https://doi.org/10.1007/978-3-662-03059-2_12

Wei Y., Zhao Y., Zhou D., Qi D., Li K., Tang W., Chen Y., Jing T., Zang X., Xie J. & Wang W. (2020). A Newly Isolated *Streptomyces* sp. YYS-7 With a Broad-Spectrum Antifungal Activity Improves the Banana Plant Resistance to *Fusarium oxysporum* f. sp. cubense Tropical Race 4. *Front. Microbiol*, 11, 1712. https://doi.org/10.3389/fmicb.2020.01712