

Rhizosphere *Streptomyces* formulas as the biological control agent of phytopathogenic fungi *Fusarium oxysporum* and plant growth promoter of soybean

MAYA SARI¹, ABDJAD ASIH NAWANGSIH², ARIS TRI WAHYUDI^{1,*}

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Agatis, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia. Tel./fax.: +62-251-8622833, *email: ariswa@apps.ipb.ac.id

²Department of Plant Protection, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Meranti, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia

Manuscript received: 3 April 2021. Revision accepted: 2 May 2021.

Abstract. Sari M, Nawangsih AA, Wahyudi AT. 2021. Rhizosphere *Streptomyces* formulas as the biological control agent of phytopathogenic fungi *Fusarium oxysporum* and plant growth promoter of soybean. *Biodiversitas* 22: 3015-3023. Rhizosphere *Streptomyces* are considered as promising sources of plant growth-promoting rhizobacteria (PGPR) and biocontrol agents against pathogenic fungi, particularly *Fusarium oxysporum* causing root rot, cotyledon rot, hypocotyl rot, and stunted growth in soybean. Formulation of rhizosphere *Streptomyces* with appropriate carrier materials is necessary to facilitate storage and application in plants. This study aimed to develop a formulation of rhizosphere *Streptomyces*, apply the formula to control *F. oxysporum*, and promote soybean plant growth. Five *Streptomyces* isolates, i.e., *Streptomyces panaciradicis* ARK 13, *Streptomyces tritolerans* ARK 17, *Streptomyces recifensis* ARK 63, *Streptomyces tendae* ARK 91, and *Streptomyces manipurensis* ARK 94 were used in this study. All of the isolates could grow in potato broth, rice bran extract, and molasses as alternative media. The highest biomass produced from the molasses growth medium. All five isolates had antifungal activity against *F. oxysporum* with the inhibition percentage ranging from 41% to 76%, and all of them were detected to have the *iaaM* gene. Indole-3-acetic acid (IAA) hormone produced by these isolates were ranging from 8.99-15.14 mg L⁻¹, with the phosphate solubilization index of 2.13-2.47. Five rhizosphere *Streptomyces* formulas with the main carrier of peat could maintain the viability with the population density of 10⁸ CFU g⁻¹ for 8 weeks of storage at room temperature. Two formulas, F17 and F94, were the best formulas to control disease caused by *F. oxysporum* with disease suppression of 74% in sterile soil and 80-85% in non-sterile soil. Formula F17 and F94 significantly increased soybean growth in sterile and non-sterile soils. Therefore, these formulas could be recommended as biocontrol and plant growth promoters of soybean.

Keywords: *Fusarium oxysporum*, growth media, rhizosphere *Streptomyces* formula, soybean

INTRODUCTION

Soil-borne fungi, particularly *Fusarium oxysporum*, are pathogens causing diseases in soybean plants. This fungus attacks at the germination phase with rot symptoms in the roots, hypocotyl, and cotyledons, causing damping-off and stunted growth therefore failure to germinate (Arias et al. 2013; Arias et al. 2013; Cui et al. 2016). *F. oxysporum* has also been reported to attack legumes (Husnain et al. 2019), peanuts (Rajeswari 2015), and beans (Ellis et al. 2013). Soybean yields could decrease by 22 kg ha⁻¹ for every 1% increase of root rot disease caused by *F. oxysporum* (Maier et al. 2018).

Pathogenic fungi are generally controlled by synthetic fungicides. However, the use of synthetic fungicides results in environmental and health problems, and microbial resistance. An alternative solution for controlling pathogenic fungi is the use of *Streptomyces* bio-agents. *Streptomyces* is Gram-positive filamentous bacteria with high G-C content so that it is easier to colonize the substrate and more resistant to environmental changes (Olanrewaju et al. 2019). *Streptomyces* can be found in various environments, such as the plant rhizosphere as PGPR. The use of indigenous microorganisms was reported

to be more effective as a biological control agent (Gopalakrishnan et al. 2011; Arora et al. 2016).

Twenty *Streptomyces* isolates from the soybean rhizosphere have been isolated in a previous study and characterized as having the ability as biocontrol and plant growth promoter agents (Mariastuti et al. 2018; Wahyudi et al. 2019). *Streptomyces* from the rhizosphere produced anti-fungal compounds that inhibit the growth of *F. oxysporum* in vitro, stimulate plant growth by producing the IAA hormone, solubilize phosphate, fix nitrogen, and increase the growth of soybean sprouts in vitro. *Streptomyces* from the rhizosphere could also produce siderophores, hydrogen cyanide, and various enzymes to degrade fungal cell walls (Glick et al. 2012; Sadeghi et al. 2012).

Five *Streptomyces* isolates from the rhizosphere, namely *Streptomyces panaciradicis* ARK 13, *Streptomyces tritolerans* ARK 17, *Streptomyces recifensis* ARK 63, *Streptomyces tendae* ARK 91, and *Streptomyces manipurensis* ARK 94 had the ability as biocontrol agents and plant growth promoters. The five potential isolates have the potential to be developed as a *Streptomyces* biocontrol formulation. *Streptomyces* biocontrol formulation is a combination of microbes with carrier

materials (Gopalakrishnan et al. 2016). The *Streptomyces* formulation from rhizosphere with an appropriate carrier material aims to facilitate the application to plants, distribution, and storage. It is necessary to formulate *Streptomyces* from the rhizosphere with a carrier material that could support the viability of *Streptomyces* during its application on plant and storage. This study aimed to formulate biocontrol *Streptomyces* from rhizosphere with the main carrier of peat, apply the *Streptomyces* biocontrol formulas to control the phytopathogenic fungus *F. oxysporum*, and promote soybean plant growth.

MATERIALS AND METHODS

Isolate preparation

Five isolates *Streptomyces* (*S. panaciradicis* ARK 13, *S. tritolerans* ARK 17, *S. recifensis* ARK 63, *S. tendae* ARK 91, and *S. manipurensis* ARK 94) from the soybean rhizosphere that has been previously isolated by Wahyudi et al. (2019) were used in this study. These isolates were re-cultured on ISP4 solid media (starch 10 g, (NH₄)₂SO₄ 2 g, CaCO₃ 2 g, K₂HPO₄ 1 g, Mg₂SO₄ 1 g, NaCl 1 g, Fe₂SO₄ 0.001 mg, MnCl₂ 0.001 mg, ZnSO₄ 0.001 mg in 1000 mL distilled water). Phytopathogenic fungus *F. oxysporum* was obtained from The Department of Plant Protection, IPB University. This fungus was re-cultured on potato dextrose agar (PDA) media for seven days.

Optimization of *Streptomyces* growth media

The alternative growth media for the production of *Streptomyces* cell biomass were potato broth (potato 200 g in 1000 mL distilled water), rice bran extract (rice bran 10 g in 1000 mL distilled water), and molasses (molasses 10 g and 3 g yeast extract in 1000 mL distilled water). Each *Streptomyces* isolate was grown on each sterilized solid growth media and incubated for seven days. Two plugs of *Streptomyces* from a solid growth medium were inoculated into 50 mL of liquid growth media. Nine tubes are provided for each liquid growth medium. After inoculation, tubes were incubated using a shaker at a speed of 100 rpm 27°C. Biomass of *Streptomyces* cells in each culture was harvested every day for nine days and centrifuged at 6000 rpm for 15 minutes at 27°C. The supernatant was filtered using filter paper and the pellets were dried in an oven at 60°C for ±18 hours. The dry biomass of *Streptomyces* in each growth media was then weighed and compared. The growth media that are capable of producing high biomass weight were used as *Streptomyces* culture media for biocontrol formulations.

Antifungal activity test

The antifungal activity assay was carried out by the dual culture method (Khare et al. 2010). The *Streptomyces* isolates were grown on molasses as the best growth medium. Seven days old *Streptomyces* from molasses solid medium were scratched on a petri dish containing PDA medium with a distance of 3 cm from the center of the plate and incubated for 48 hours at 27°C. The fungus *F. oxysporum* with a diameter of 8 mm from PDA medium

was taken and placed in the center of the plate, facing *Streptomyces* isolates. Incubation was continued for seven days at 27°C. The percentage of inhibition was calculated after seven days of incubation using the equation as follows:

$$\text{Percentage of inhibition (\%)} = \frac{R_1 - R_2}{R_1}$$

Where; R₁: growth radius of *F. oxysporum* in the negative control/without *Streptomyces* (cm), R₂: growth radius of *F. oxysporum* on the opposite side of *Streptomyces* isolate (cm)

Detection of *iaaM* gene in *Streptomyces*

Five *Streptomyces* isolates were grown on a molasses solid medium for seven days. The *Streptomyces* DNA was extracted using The Genomic DNA Mini Kit. The DNA was amplified using a polymerase chain reaction (PCR). The size of the detected gene was 1698 base pairs, using primer *iaaM_F* (5'-ATG ACG TCC ACC GTG CCC AAC GCG-3') and *iaaM_R* (5'-CTA GTC CTC GGG GAG TTC CAC GGG-3'). The PCR condition was preceded by pre-denaturation (94°C, 5 minutes), denaturation (95°C, 40 seconds), annealing (59°C, 30 seconds), elongation (72°C, 2 minutes), and post elongation (72°C, 5 minutes) (Lin and Xu 2013). The PCR process was carried out for 27 cycles and ended with post PCR (4°C, 5 minutes). The amplification results were then sent to the sequencing service for similarity sequences. The search for similarity sequences was done using the Basic Local Alignment Search Tool (BLAST) program, available at the National Center for Biotechnology Information (NCBI).

IAA production

Measurement of IAA production was carried out using the colorimetric method (Khamna et al. 2010). Each isolate was cultured in 20 mL of molasses medium with the addition of 0.2 mL of L-tryptophan 0.2%. The cultures were then incubated in a shaker incubator at 100 rpm 27°C for eight days. After incubation complete, the culture was centrifuged at 11.000 ppm, 4°C for 15 minutes. One mL of supernatant was taken from each culture and mixed with 4 mL of Salkowski reagent (FeCl₃·6H₂O 0.5% 7.5 mL and H₂SO₄ 150 mL in 250 mL distilled water), then incubated in a dark room for 30 minutes. The absorbance of the mixture was measured using a Spectrometer at a wavelength of 520 nm. The IAA concentration produced by each isolate was calculated by comparing the absorbance values on the IAA standard curve.

Phosphate solubilization test

The phosphate solubilization test was carried out by inoculating an agar plug (8 mm diameter) of seven days old *Streptomyces* on Pikovskaya medium (glucose 10 g, Ca₃(PO₄) 5 g, (NH₄)₂SO₄ 0.5 g, MgSO₄·2H₂O 0.1 g, MnSO₄ 0.1 g, FeSO₄ 0.1 g, yeast extract 0.1 g, and agar 15 g in 1000 mL distilled water), and then incubated for seven days at 27°C. *Streptomyces* isolates that could form a clear zone indicate phosphate dissolving activity. The phosphate solubilization index then calculated using the equation as follows:

$$\text{The phosphate solubilization index} = \frac{\text{Colony diameter} + \text{Clear zone diameter}}{\text{Colony diameter}}$$

Streptomyces formulation

The formulation used is in the form of powder with the main carrier material is peat. It was obtained from The Indonesian Institute for Biotechnology and Bio-Nutrition Research, Bogor, West Java. The carrier material consisted of 85% peat, 10% rock phosphate, and 5% CaCO₃ that was packed into 10 g of heat-resistant plastic and sterilized twice using an autoclave. *Streptomyces* isolates were cultured on molasses medium for seven days. Five mL of *Streptomyces* culture (10¹⁰ CFU mL⁻¹) was inoculated using a sterile syringe into 10 g of the sterile carrier material. The formulation was then incubated at room temperature (27°C). The viability of *Streptomyces* in the formulation was observed using the Total Plate Count (TPC) method and the moisture content of each formulation was measured by comparing the wet and dry weights.

Application of the formula on soybean plant

The application of the biocontrol formulation on soybean plant was performed in several steps as described below.

Experiment design in greenhouse

The test of *Streptomyces* formula on soybean was performed at Cikabayan Experimental Garden, IPB University. The experimental design of the study was a Completely Randomized Design. There were 3 treatments, namely (i) seeds coated with *Streptomyces* formula, planted on sterile and non-sterile soil infested with *F. oxysporum*, (ii) seeds without *Streptomyces* formula coatings, planted on sterile and non-sterile soil infested with *F. oxysporum* (positive control), (iii) seeds without *Streptomyces* formula coatings, planted on sterile and non-sterile soil that was not infested with *F. oxysporum* (negative control). Each treatment has 3 replications and each replication consisting of ten soybean seeds.

Soil preparation

The soil composition used was ultisol soil, sand soil, and compost with a ratio of 2:1:1. The soil treatment used was sterile and non-sterile soils. The soil sterilization was carried out twice using an autoclave. Sterile and non-sterile soils were then inserted into the trays (45 holes per tray).

Preparation of the fungal pathogen F. oxysporum

The fungal pathogen *F. oxysporum* was grown on PDA media for seven days. One plug (8 mm) of seven-day-old *F. oxysporum* was inoculated into 100 mL of potato dextrose broth (PDB) media. The cultures were incubated using a shaker incubator at 100 rpm 27°C for seven days. The culture concentration was then determined by the TPC method. A total of 30 mL of *F. oxysporum* (10³ CFU mL⁻¹) was infested into sterile and non-sterile soils in each tray hole.

Preparation of soybean seeds

The variety of soybean seeds used in this study was the Grobogan variety. Soybean seeds were surface sterilized by

immersing the seeds in 96% ethanol for 5 minutes. The seeds were then soaked in 2.5% NaOCl for 1 minute and rinsed ten times with sterile distilled water. Sterile seeds were soaked in sterile distilled water for 24 hours to accelerate germination. The seeds were then coated with each *Streptomyces* formula using the seed coating method and sown in 1 cm from the soil surface.

Observation of disease incidence and disease suppression

The observation of disease incidence and disease suppression in soybean was calculated after seven days of sowing. Symptoms of the disease observed were rot of root, hypocotyl, and cotyledon; stunted growth, and failure to germinate. The percentage of disease incidence was calculated using the equation:

$$\text{Disease incidence (\%)} = \frac{n}{N} \times 100$$

Where; n: number of symptomatic plants, N: total plants observed

The percentage of disease suppression was then calculated using the equation:

$$\text{Disease suppression (\%)} = \frac{(X - C^+)}{(C^- - C^+)} \times 100$$

Where; X: number of healthy plants in each treatment, C⁺: number of healthy plants in the non-coating treatment planted on soil infested with pathogenic fungi, C⁻: number of healthy plants in the non-coating treatment planted on soil that was not infested with pathogenic fungi

Observation of plant growth

The seven-day-old soybean plant was uprooted and cleaned from soil and dirt. Growth parameters observed were root length, plant length, wet weight, and dry weight. The dry weight was determined by drying the plants in an oven at 60°C for 24 hours.

Data analysis

Data on disease incidence, disease suppression, and growth promoters were statistically analyzed using one-way analysis of variance (ANOVA). The analysis was continued with the Tukey test with a confidence level of 95%. All data were analyzed using the SPSS version 25 application.

RESULTS AND DISCUSSION

Optimization of Streptomyces growth media

Five *Streptomyces* isolates grew well on all types of solid growth media. The cell biomass of *Streptomyces* was varied in each growth medium (Figure 1). Molasses is the medium that produces the highest cell biomass of *Streptomyces* for five *Streptomyces*. The weight of cell biomass of *S. recifensis* ARK 63 in molasses medium was 2.94 mg mL⁻¹ at seven days of incubation.

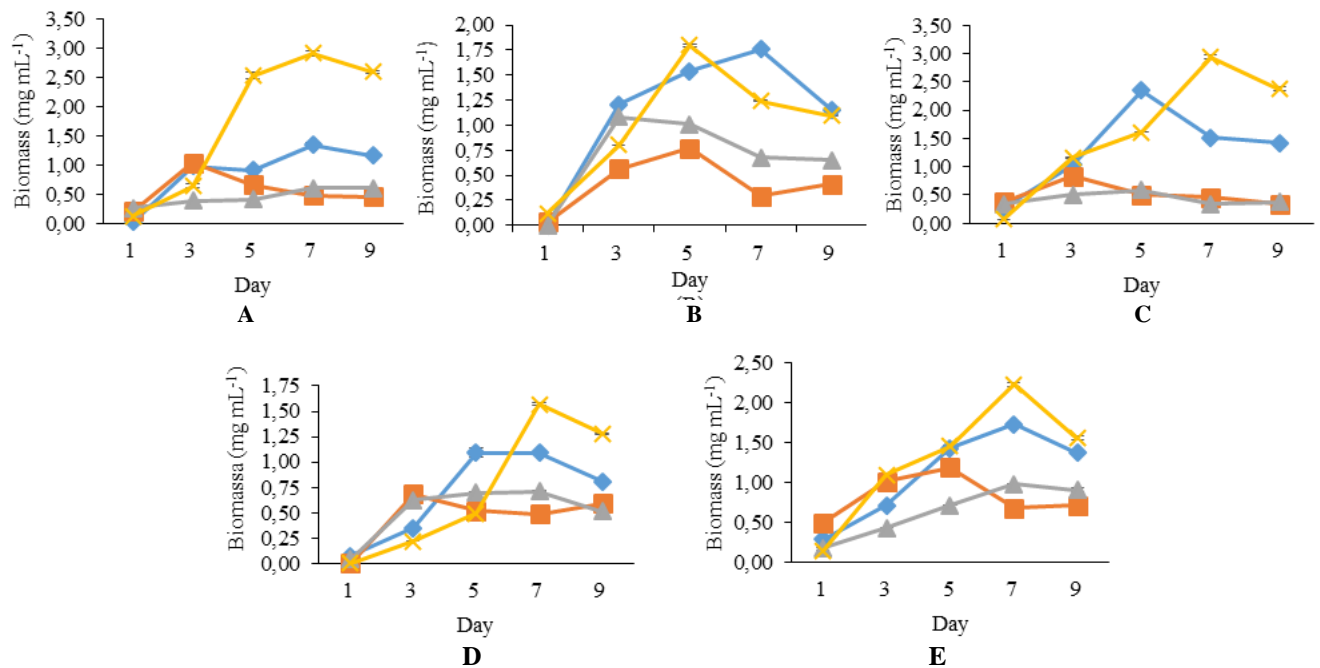


Figure 1. The curves of *Streptomyces* biomass. A. *S. panaciradicis* ARK 13, B. *S. tritolerans* ARK 17, C. *S. recifensis* ARK 63, D. *S. tendae* ARK 91, and E. *S. manipurensis* ARK 94 on growth media of (—◆—) ISP4, (—■—) potato broth, (—▲—) bran extract, and (—×—) molasses

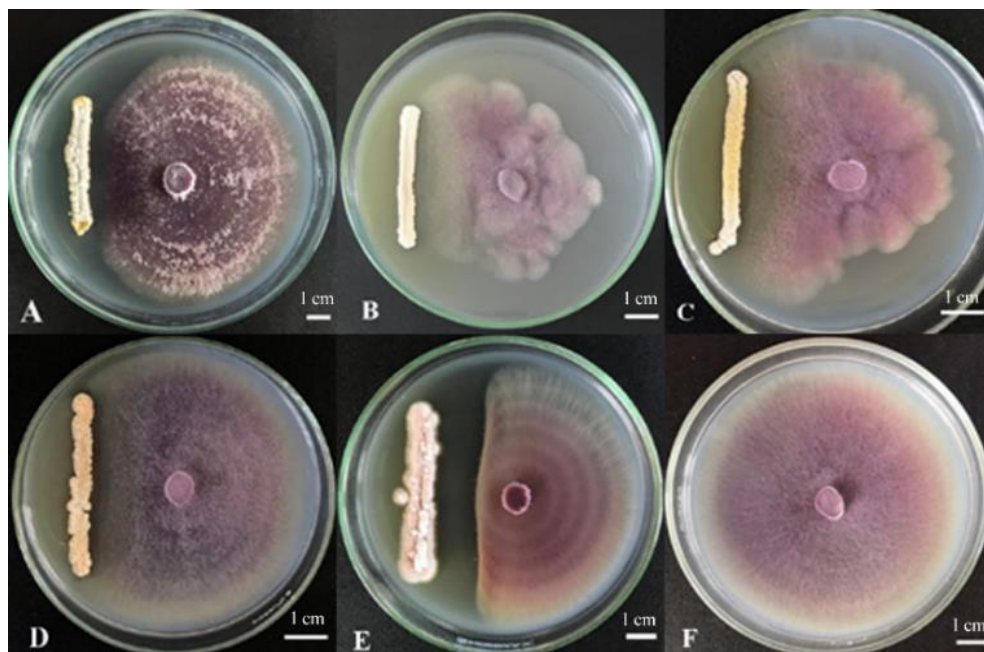


Figure 2. The growth inhibition of the pathogenic fungi *F. oxysporum* by *Streptomyces* isolates on PDA media after seven days incubation. A. *S. panaciradicis* ARK 13, B. *S. tritolerans* ARK 17, C. *S. recifensis* ARK 63, D. *S. tendae* ARK 91, E. *S. manipurensis* ARK 94, and F. control *F. oxysporum*.

Antifungal activity

The growth inhibition of *F. oxysporum* by five *Streptomyces* isolates was varied (Table 1). The highest inhibition after seven days of incubation was produced by *S. manipurensis* ARK 94, with an inhibition percentage of 76%. The growth of *F. oxysporum* mycelium on the

opposite of the *Streptomyces* isolates was inhibited compared to the growth of *F. oxysporum* mycelium on control (Figure 2). The growth inhibition of *F. oxysporum* might be due to the anti-fungal bioactive compounds produced by *Streptomyces* isolates. Microscopic observations were made on *S. manipurensis* ARK 94 as the

best inhibitor of *F. oxysporum* mycelium growth. The morphology of hyphal *F. oxysporum* inhibited by *S. manipurensis* ARK 94 indicated hyphal lysis and granulation (Figure 3).

Detection of *iaaM* gene in *Streptomyces*

The amplification results using specific primers showed that the five *Streptomyces* isolates produced a target band measuring ± 1698 bp (Figure 4). Based on the BLASTX analysis, the bands were identified as Tryptophan-2-monooxygenase. *Streptomyces* spp. have varied similarity levels between 85.5% and 96.6% (Table 2). The phylogenetic tree was formed by the Neighbor-Joining method (Figure 5).

IAA production

Five *Streptomyces* isolates were able to produce IAA with varying concentrations of 8.99 to 15.14 mg L⁻¹ (Table 3). The highest IAA concentration was produced by *S. tendae* ARK 91 isolates.

Phosphate solubilization

The ability to solubilize phosphate was characterized by the formation of a clear zone around the colony on Pikovskaya media. All of the isolates produced a clear zone around the colony and were able to solubilize phosphate with a phosphate solubilizing index of 2.13 to 2.47 (Table 4). The highest phosphate solubilizing index was produced by *S. manipurensis* ARK 94.

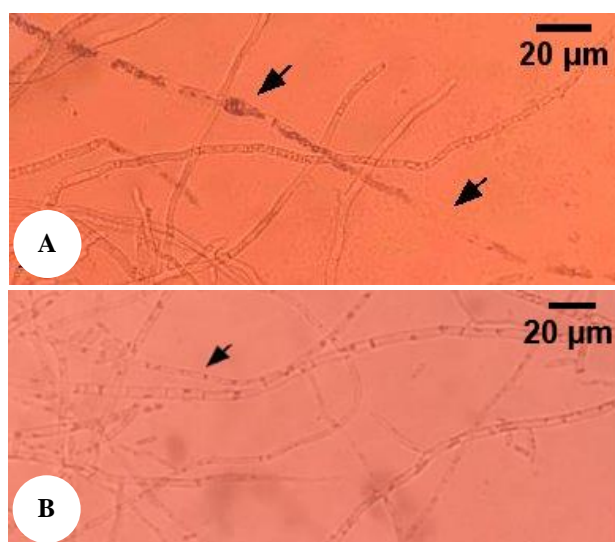


Figure 3. A. Morphology of *F. oxysporum* hyphae against *S. manipurensis* ARK 94, B. Normal hyphae of *F. oxysporum*

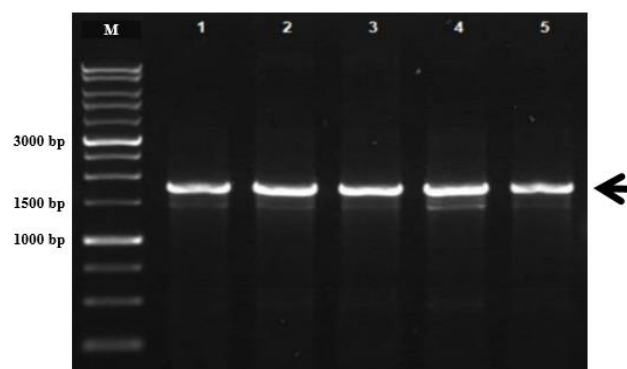


Figure 4. Visualization of the *iaaM* gene (± 1698 bp, arrowhead) of *Streptomyces* isolates on 1% agarose gel. (M) marker, (1) *S. panaciradicis* ARK 13, (2) *S. tritolerans* ARK 17, (3) *S. recifensis* ARK 63, (4) *S. tendae* ARK 91, and (5) *S. manipurensis* ARK 94

Table 1. The growth inhibition of *F. oxysporum* by *Streptomyces* isolates on PDA after seven days of incubation.

Isolates	Growth inhibition (%) \pm SD
<i>S. panaciradicis</i> ARK 13	51 \pm 0.00
<i>S. tritolerans</i> ARK 17	41 \pm 2.10
<i>S. recifensis</i> ARK 63	52 \pm 1.11
<i>S. tendae</i> ARK 91	45 \pm 7.55
<i>S. manipurensis</i> ARK 94	76 \pm 2.10

Table 2. The IAA production of *Streptomyces* isolates on molasses culture medium with the addition of L-tryptophan 0.2%

Isolates	IAA concentration (mg L ⁻¹) \pm SD
<i>S. panaciradicis</i> ARK 13	12.25 \pm 0.64
<i>S. tritolerans</i> ARK 17	13.39 \pm 0.23
<i>S. recifensis</i> ARK 63	08.99 \pm 0.23
<i>S. tendae</i> ARK 91	15.14 \pm 0.14
<i>S. manipurensis</i> ARK 94	13.44 \pm 0.18
<i>Bradyrhizobium japonicum</i> (control)	16.24 \pm 0.05

Table 3. Phosphate solubilization index of *Streptomyces* isolates on Pikovskaya medium incubated for seven days.

Isolates	Phosphate solubilization index \pm SD
<i>S. panaciradicis</i> ARK 13	2.43 \pm 0.19
<i>S. tritolerans</i> ARK 17	2.23 \pm 0.09
<i>S. recifensis</i> ARK 63	2.13 \pm 0.31
<i>S. tendae</i> ARK 91	2.33 \pm 0.05
<i>S. manipurensis</i> ARK 94	2.47 \pm 0.09

Table 4. The diversity of *iaaM* genes of *Streptomyces* isolates by BLASTX.

Isolates	Homology	Query cover (%)	Similarity (%)	Accession number
<i>S. panaciradicis</i> ARK 13	Tryptophan 2-monooxygenase [<i>S. paradoxus</i>]	99	95.1	MBB6078269.1
<i>S. tritolerans</i> ARK 17	Tryptophan 2-monooxygenase [<i>Streptomyces</i> sp. MH60]	96	85.5	PPS84631.1
<i>S. recifensis</i> ARK 63	Tryptophan 2-monooxygenase [<i>Streptomyces</i> sp. 2221.1]	95	96.6	REH19738.1
<i>S. tendae</i> ARK 91	Tryptophan 2-monooxygenase [<i>Streptomyces</i> sp. BK208]	95	96.1	TDT36864.1
<i>S. manipurensis</i> ARK 94	Tryptophan 2-monooxygenase [<i>Streptomyces</i> sp. SLBN-109]	99	94.5	TQK33573.1

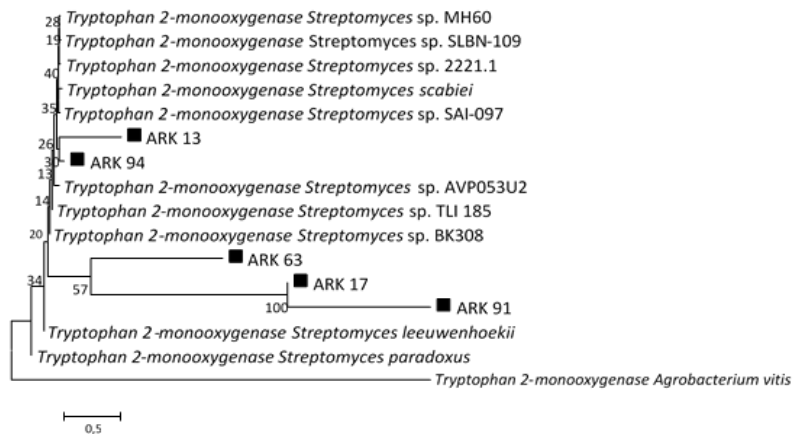


Figure 5. Phylogenetic tree showing the relationship between the five *Streptomyces* isolates tryptophan-2-monoxygenase with the comparison species using the Neighbor-Joining method based on the *iaaM* gene sequence. The black box was the *Streptomyces* isolate used. The number 0.5 showed the kinship scale. Tryptophan-2-monoxygenase *Agrobacterium vitis* was used as the outgroup

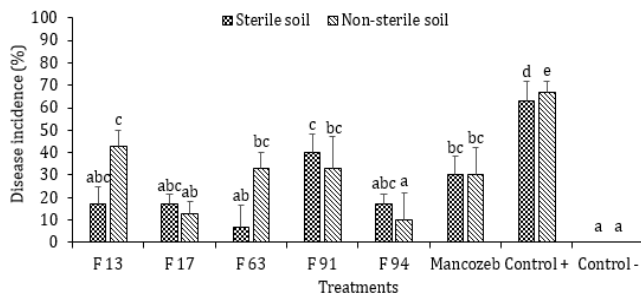


Figure 6. The percentage of disease incidence caused by *F. oxysporum* in each treatment.

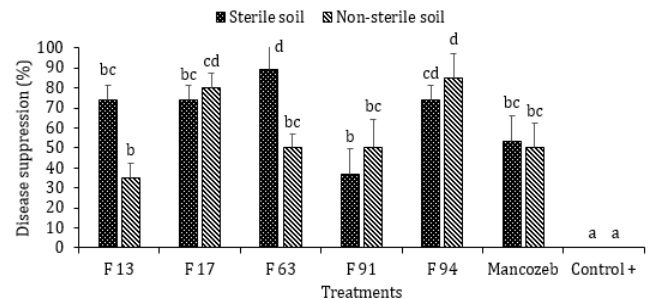


Figure 7. The percentage of disease suppression in each treatment

The viability of *Streptomyces* formulas

Five *Streptomyces* formulas were able to maintain viability at week 8 with an average of 10^8 CFU g^{-1} . The highest concentration was found at F91, which was 10×10^8 CFU g^{-1} . Meanwhile, the lowest concentration was found at F13, which was 5×10^7 CFU g^{-1} . The moisture content of each formula stored at room temperature varied from 44% to 52%. The highest water content was at F13 and the lowest water content was at F94. The measurement of pH was also carried out for each type of *Streptomyces* formula. All the formulas had a pH of 7.

Application of *Streptomyces* formulas on soybean plant

Fusarium oxysporum infestation caused rot of root, hypocotyl, cotyledon, and damping-off in soybean plants. The results of statistical tests showed that the five *Streptomyces* formulas reduced disease incidence and increase disease suppression significantly in soybean plants. The lowest disease incidence (7%) was found in F63 in sterile soil and F94 (10%) in non-sterile soil (Figure 6). The highest disease suppression was found in F63 (89%) in sterile soil and F94 (85%) in non-sterile soil (Figure 7). Four formulas (F13, F17, F63, and F94) on sterile soil and two formulas (F17 and F94) on non-sterile soil were able to decrease disease incidence and increase disease incidence better than synthetic fungicide Mancozeb, and significantly increased soybean plant growth (Table 5). F17 and F94 were the best

formulas for suppressing pathogenic fungal disease of *F. oxysporum* and increasing soybean growth.

Table 5. The growth of seven-day-old soybean plants in sterile and non-sterile soil infested with *F. oxysporum*.

Treatments	Growth parameters			
	Root length (cm)	Plant length (cm)	Wet weight (g)	Dry weight (g)
Sterile soil				
Control +	3.44 ab	7.83 ab	0.60 ab	0.09 ab
Control -	4.92 a	10.67 a	0.71 a	0.11 a
F13	8.92 cd*	14.31 c*	1.22 c*	0.22 c*
F17	8.63 bcd	13.21 bc	1.24 c*	0.27 c*
F63	8.42 bcd	13.55 bc	0.90 abc	0.17 abc
F91	9.34 d*	13.47 bc	1.17 c*	0.20 bc
F94	8.98 cd*	13.96 c*	1.11 bc	0.17 abc
Mancozeb	5.17 abc	11.59 bc	0.93 abc	0.16 abc
Non-sterile soil				
Control +	2.96 a	9.13 a	0.49 a	0.12 a
Control -	3.42 ab	10.21 ab	0.65 ab	0.11 a
F13	5.25 ab	13.71 bc	0.82 bc	0.15 ab
F17	6.38 b	14.84 c*	1.15 d*	0.25 b*
F63	5.63 ab	12.84 bc	0.90 bc	0.15 ab
F91	5.30 ab	13.13 bc	0.90 bcd	0.15 ab
F94	6.21 b	14.75 c*	0.99 cd*	0.14 ab
Mancozeb	5.17 ab	10.78 abc	0.81 bc	0.11 a

Note: *: The growth parameters were significantly increased and different than the negative control at the 95% confidence level

Discussion

Five *Streptomyces* isolates were successfully cultured on alternative growth media. *Streptomyces* was chemoorganotrophic which could grow on various types of carbon sources, including monosaccharides, disaccharides, polysaccharides, polyols, and various organic acids (Sousa et al. 2016). The results showed that molasses medium could produce higher cell biomass of *Streptomyces*. The molasses was a byproduct of the sugar cane processing into sugar. The molasses contain 90% sucrose and 10% glucose (Basso et al. 2011). Sucrose was a disaccharide from the monomers glucose and fructose which could be used by *Streptomyces* as a carbon source. The simpler carbon sources in molasses can be used more easily by *Streptomyces* compared to starch as the more complex carbon sources in potato broth and bran extract growth media. The addition of yeast extract as a source of organic nitrogen was also reported to affect the cell biomass production of *Streptomyces*. Saurav et al. (2010) reported that the addition of organic nitrogen sources such as yeast to culture media yield relatively high cell biomass of *Streptomyces*. The use of molasses as a growing medium increased the productivity of antimicrobial compounds in *Streptomyces cyaneus* DN 37 (Abdelwahed et al. 2012). It indicates that the molasses medium might be used as a growth medium for the production of *Streptomyces* biomass.

The optimum incubation time in the molasses medium was similar to the ISP4 media (day 5th and 7th). *Streptomyces* biomass reaches its optimum weight under optimal conditions for mycelium formation. The life cycle of *Streptomyces* starts in an exponential phase when the spores germinate into vegetative hyphae then grow to form branched hyphae or vegetative hyphae. The decrease in biomass that occurred on the 9th day might be due to decreased nutrients in the media. In nutrient deficiency conditions, vegetative hyphae was autolysis degraded by cell death mechanism to form aerial hypha, and antibiotics were produced in this phase (Barka et al. 2016).

The growth of *F. oxysporum*, as opposed to *Streptomyces*, was inhibited without direct contact. This suggests that the five isolates grown on molasses media could still inhibit the growth of the pathogenic fungi *F. oxysporum*. It indicated that *Streptomyces* produced bioactive compounds that might have anti-fungal activity and cell wall degrading enzymes. Microscopic observations showed the presence of lysis and granulation of *F. oxysporum* hyphae. *Streptomyces indiaensis* SRT1 produces secondary metabolites as an antifungal which inhibits spore germination, hyphal segmentation, and distortion in spores of *F. oxysporum* NCIM 1072 (Bhosle et al. 2018). The chitinase enzyme produced by *S. griseorubens* E44G from soil could inhibit *F. oxysporum* which causes wilt on tomatoes (Rashad et al. 2017). *Streptomyces spororaveus* RDS28 grown on simple carbon source media could produce optimal antifungal compounds capable of inhibiting the growth of *Rhizoctonia solani*, *F. solani*, *F. verticillioides*, *Alternaria alternata*, and *Botrytis cinerea* (Al-Askar et al. 2011).

The detection of the *iaaM* gene in five isolates indicated that these isolates might be able to produce IAA using the indole-3 acetamide (IAM) pathway. Two enzymes play a role in the IAM pathway, namely tryptophan-2-monooxygenase and indole-3-acetamide hydrolase. The former enzyme converts the amino acid tryptophan into intermediate IAM and the later enzyme hydrolyzed IAM into IAA. Based on the results of BLASTX, the *iaaM* genes in five isolates had a similarity level of 85.5% to 96.6% with tryptophan-2-monooxygenase *Streptomyces* spp. The result confirmed the result of the previous study, that five isolates were able to produce IAA hormone and increase the growth of soybean plants in vitro (Wahyudi et al. 2019). Several previous studies reported that the *Streptomyces* spp. have been known to have the gene encoding *iaaM*. Passari et al. (2016) reported that *Streptomyces* spp. associated with tomatoes has the *iaaM* gene. Generally, rhizobacteria were able to synthesize IAA through the IAM pathway (Zhang et al. 2019).

Five isolates were able to produce IAA in molasses medium with the addition of L-tryptophan 0.2%. Supplementation of 0.2% L-tryptophan in the growth media was aimed to get the optimum condition for the production of IAA by *S. fradiae* NKZ-259 (Myo et al. 2019). The difference in IAA production of five *Streptomyces* in this study could be caused by differences in the metabolic ability of the isolate to convert L-tryptophan to IAA. A previous study by Wahyudi et al. (2019) showed that IAA production of *Streptomyces* from soybean rhizosphere cultured on media with simple carbon, organic nitrogen, and the addition of 0.2% L-tryptophan was in the range of 2.08 to 16.70 mg L⁻¹. It was assumed that five *Streptomyces* isolates were able to produce IAA in molasses growth medium.

All of the isolates were able to solubilize phosphate in Pikovskaya media with Ca₃(PO₄) as the phosphate source. These isolates might produce organic acids which are capable of chelating Ca from Ca₃(PO₄). A study by Farried et al (2018) showed that *Streptomyces* spp. isolated from the rhizosphere could produce organic acids that solubilize phosphate with the solubilizing index of 1.57 to 3.83. *Streptomyces* isolated from wheat roots produced malic acid which can solubilize phosphate and increased wheat growth (Jog et al. 2014).

The *Streptomyces* formulas were able to maintain viability with the population density of 10⁸ CFU g⁻¹ at 8 weeks and water content of 44% to 52%. The bacteria in bacterial formula with peat carrier material survives up to 180 days with a population density of 10⁸ CFU g⁻¹ (Khabbaz and Abbasi 2014). Water content also affects the survival of microbes in the peat carrying material by 40% to 50% (Casteriano et al. 2013).

Formulas of F17 and F94 could significantly suppress the disease caused by *F. oxysporum* and promote the growth of soybean plants. *Streptomyces* suppresses disease incidence by antibiotic mechanisms, hyperparasitism, produce various bioactive compounds and enzymes that degrade fungal cell walls (Khalil et al. 2014). The formulated cells of *S. griseus* could inhibit the growth of *F. oxysporum* f.sp. *cubense* on sterile soil (Zacky and Ting

2015). *Streptomyces* ma. *Fs-4* could reduce the disease incidence caused by *Fusarium* and increase banana growth (Duan et al. 2020). The powder formulation of *S. corchorusii* strain UCR3-16 also increases growth significantly in rice (Tamreihao et al. 2016).

Based on the results of the study, the *Streptomyces* formulas with the peat carrying material can maintain viability up to 8 weeks with a population density of 10^8 CFU g^{-1} at room temperature. Formulas F17 and F94 were the best formulas to control disease caused by *F. oxysporum* with the disease suppression of 74% in sterile soil and 80-85% in non-sterile soil. These formulas also significantly increased the growth of soybean plants in sterile and non-sterile soils in the greenhouse experiment.

ACKNOWLEDGEMENTS

Part of this work was funded by The Ministry of Research and Technology/ National Research and Innovation Agency of The Republic of Indonesia in 2020, and partly was funded in 2021 through “*Penelitian Dasar-Unggulan Perguruan Tinggi*” (PD-UPT) to ATW (Contract No. 1/E1/KP.PTNBH/2020; and No. 1/E1/KP.PTNBH/2021). Therefore, we thank and appreciate all the supports given to carry out this research.

REFERENCES

- Abdelwahed NAM, Abdallah NA, El-Ghawas DE, El-Din SMB, El-Diwany AI. 2012. Isolation, identification and optimization of antimicrobial metabolites produced by soil-derived actinomycetes. *Egypt J Exp Biol* 8 (2): 205-217.
- Al-Askar AA, Abdulaziz KMW, Rashad YM. 2011. In vitro antifungal activity of *Streptomyces spororaveus* RDS28 against some phytopathogenic fungi. *Afr J Agri Res* 6 (12): 2835-2842.
- Arias MMD, Leandro LF, Munkvold GP. 2013. Aggressiveness of *Fusarium* species and impact of root infection on growth and yield of soybeans. *Ecol Epidemiol* 8 (103): 822-832. DOI: 10.1094/phyto-08-12-0207-r.
- Arias MMD, Munkvold GP, Ellis ML, Leandro LES. 2013. Distribution and frequency of *Fusarium* species associated with soybean roots in Iowa. *Plant Dis* 97: 1157-1562. DOI: 10.1094/PDIS-11-12-1059-RE.
- Arora NK, Mishra J. 2016. Prospecting the roles of metabolites and additives in future bioformulations for sustainable agriculture. *Appl Soil Ecol* 107: 405-407. DOI: 10.1016/j.apsoil.2016.05.020.
- Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H-P, Clément C, Ouhdouch Y, Wezel GP. 2016. Taxonomy, physiology, and natural products of actinobacteria. *Microbiol Mol Biol Rev* 80: 1-43. DOI: 10.1128/MMBR.00019-15.
- Basso LC, Basso TO, Rocha SN. 2011. Ethanol production in Brazil: the industrial process and its impact on yeast fermentation. *Biofuel Prod-Rec Dev Pros* 1530: 85-100. DOI: 10.5772/17047.
- Bhosle HJ, Kadam TA, Mirajgave RS, Holkar SK. 2018. Optimization and characterization of antifungal metabolite from a soil actinomycete *Streptomyces indiaensis* SRT. *Indian J Biotechnol* 17 (2): 261-271.
- Casteriano A, Wilkes MA, Deaker R. 2013. Physiological changes in rhizobia after growth in peat extract may be related to improved desiccation tolerance. *Appl and Environ Microbiol* 79 (13): 3998-4007. DOI: 10.1128/AEM.00082-13.
- Cui J, Wang Y, Han J, Cai B. 2016. Analyses of the community compositions of root rot pathogenic fungi in the soybean rhizosphere soil. *Chil J Agr Res* 76 (2): 179-187. DOI: 10.4067/S0718-58392016000200007.
- Duan Y, Chen J, He W, Chen J, Pang Z, Hu H, Xie J. 2020. Fermentation optimization and disease suppression ability of a *Streptomyces* ma. *Fs-4* from banana rhizosphere soil. *BMC Microbiol* 20 (24): 1-12. DOI: 10.1186/s12866-019-1688-z.
- Ellis ML, Arias MMD, Jimenez DRC, Munkvold GP, Leandro LF. 2013. First report of *Fusarium commune* causing damping-off, seed rot, and seedling root rot on soybean (*Glycine max*) in the United States. *Plant Dis* 97 (2): 284. DOI: 10.1094/PDIS-07-12-0644-PDN.
- Farried, Al-Shaimaa M, Mohamed HM, El-Dsouky, El-Rewainy HM. 2018. Isolation and characterization of phosphate solubilizing actinomycetes from rhizosphere soil. *Assiut J Agric Sci* 49 (4): 125-137.
- Glick BR. 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*. DOI: 10.6064/2012/963401.
- Gopalakrishnan S, Pande S, Sharma M, Humayun P, Kiran BK, Sandeep D, Vidya MS, Deepthi K, Rupela O. 2011. Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of *Fusarium* wilt of chickpea. *Crop Prot* 30 (8): 1070-1078. DOI: 10.1016/j.cropro.2011.03.006.
- Gopalakrishnan S, Sathya A, Vijayabharathi R, Srinivas V. 2016. Formulation of plant growth-promoting microbes for field applications. *Microb Inocul Sustain Agric Product* 15: 239-251. DOI: 10.1007/978-81-322-2644-4_15.
- Husnain SK, Khan SB, Atiq M, Rajput NA, Abbas W, Mohsin M. 2019. Screening of peas (*Pisum sativum*) varieties/ lines against fusarium wilt (*Fusarium oxysporum* f. sp. *Pisi*) and in vitro evaluation of fungicides against mycelial growth of pathogen. *Pak J Phtopathol* 31 (1): 89-96. DOI: 10.33866/phytopathol.031.01.0492.
- Jog R, Pandya M, Narshkumar G, Rajkumar S. 2014. Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. *Microbiology* 160: 778-788. DOI: 10.1099/mic.0.074146-0.
- Khabbaz SE, Abbasi PA. 2014. Isolation, characterization, and formulation of antagonistic bacteria for the management of seedlings damping-off and root rot disease of cucumber. *Can J Microbiol* 60 (1): 25-33. DOI: 10.1139/cjm-2013-0675.
- Khalil MS, Moubasher H, Hasan FF. 2014. Biological control of rice blast disease by *Streptomyces flavotricini*. *Res J Pharm Biol Chem Sci* 5: 1453-1461.
- Khamna S, Yokota A, Peberdy JF, Lumyong S. 2010. Indole-3-acetic acid production by *Streptomyces* sp. isolated from Thai medicinal plant rhizosphere soils. *EurAsia J BioSci* 4: 23-32. DOI: 10.5053/ejobios.2010.4.0.4.
- Khare A, Singh BK, Upadhyay RS. 2010. Biological control of *Pythium aphanidermatum* causing damping-off of mustard by mutants of *Trichoderma viridae* 1433. *J Agric Technol* 6: 231-243.
- Lin L, Xu X. 2013. Indole-3-acetic acid production by endophytic *Streptomyces* sp. En-1 isolated from medical plants. *Curr Microbiol* 67: 209-217. DOI: 10.1007/s00284-013-0348-z.
- Maier M, Souza CA, Casa RT. 2018. Cropping systems on root rot and soybean seed yield. *Ciência Rural* 48 (7): 1-8. DOI: 10.1590/0103-8478cr20170460.
- Mariastuti HD, Listiyowati S, Wahyudi AT. 2018. Antifungal activity of soybean rhizosphere actinomycetes producing bioactive compounds against *Fusarium oxysporum*. *Biodiversitas* 19 (6): 2127-2133. DOI: 10.13057/biodiv/d190619.
- Myo EM, Ge B, Ma J, Cui H, Liu B, Shi L, Jiang M, Zhang K. 2019. Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth. *BMC Microbiol* 19 (155): 1-14.
- Olanrewaju OS, Babalola OO. 2019. *Streptomyces*: implications and interactions in plant growth promotion. *Appl Microbiol Biotechnol* 103: 1179-1188. DOI: 10.1007/s00253-018-09577-y.
- Passari AK, Chandra P, Zothanpuia, Mishra VK, Leo VV, Gupta VK, Kumar B, Singh BP. 2016. Detection of biosynthetic gene and phytohormone production by endophytic actinobacteria associated with *Solanum lycopersicum* and their plant-growth-promoting effect. *Res Microbiol* 167 (8): 692-705. DOI: 10.1016/j.resmic.2016.07.001.
- Rajeswari P. 2015. Control of *Fusarium oxysporum* causing fusarium wilt by *Trichoderma* spp and *Pseudomonas fluorescens* on *Arachis hypogaea* L. *Int J Adv Biotechnol Res* 6: 57-65.
- Rashad, Younes, Al-Askar, Abdulaziz, Ghoneem, Khalid, Ali S, Wesameldin, Hafez, Elsayed. 2017. Chitinolytic *Streptomyces griseorubens* E44G enhances the biocontrol efficacy against *Fusarium* wilt disease of tomato. *Phytoparasitica* 45 (2): 227-237. DOI: 10.1007/s12600-017-0580-3.

- Sadeghi A, Karimi E, Dahaji PA, Javid MG, Dalvand Y, Askari H. 2012. Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. *World J Microbiol Biotechnol* 28: 1503-1509. DOI: 10.1007/s11274-011-0952-7.
- Saurav K, Kannabiran K. 2010. Diversity and optimization of process parameters for the growth of *Streptomyces* VITSVK9 spp. isolated from Bay of Bengal, India. *J Nat Env Sci* 1 (2): 56-65.
- Sousa JADJ, Olivares FL. 2016. Plant growth promotion by *Streptomyces*: Ecophysiology, mechanisms and applications. *Chem and Biol Technol Agric* 3 (24): 1-12. DOI: 10.1186/s40538-016-0073-5.
- Tamreihao K, Ningthoujam DS, Nimaichand S, Singh ES, Reena P, Singh SH, Nongthomba U. 2016. Biocontrol and plant growth promoting activities of a *Streptomyces corchorusii* strain UCR3-16 and preparation of powder formulation for application as biofertilizer agents for rice plant. *Microbiol Res* 192: 260-270. DOI: 10.1016/j.micres.2016.08.005.
- Wahyudi AT, Priyanto JA, Afrista R, Kurniati D, Astuti RI, Akhdiya A. 2019. Plant growth promoting activity of actinomycetes isolated from soybean rhizosphere. *Online J Biol Sci* 19 (1): 1-8. DOI: 10.3844/ojbsci.2019.1.8.
- Zacky FA, Ting ASY. 2015. Biocontrol of *Fusarium oxysporum* f.sp. *cubense* tropical race 4 by formulated cells and cell-free extracts of *Streptomyces griseus* in sterile soil environment. *Biocontrol Sci Technol* 25 (6): 685-696. DOI: 10.1080/09583157.2015.1007921.
- Zhang P, Jin T, Kumar Sahu S, Xu J, Shi Q, Liu H, Wang Y. 2019. The distribution of tryptophan-dependent indole-3-acetic acid synthesis pathways in bacteria unraveled by large-scale genomic analysis. *Molecules* 24 (7): 1-14. DOI: 10.3390/molecules2407141.