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Harnessing *Serratia marcescens*: A Dual Role as a Biocontrol Agent Against *Fusarium oxysporum f.sp. melongenae* and Heteroauxin Producer

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Abstract: Pathogens and plant growth factors significantly influence plant growth and development. This study aims to investigate the potential of *Serratia marcescens* as a biocontrol agent against *Fusarium oxysporum f.sp. melongenae* and its ability to create heteroauxin. Antagonistic tests of *S. marcescens* against *F. oxysporum f.sp. melongenae* were conducted using a dual culture method while the antifungal activity of *S. marcescens* extract was assessed through the disk diffusion method. Salkowski's colorimetric test was performed both in vitro and in situ to analyze heteroauxin compounds, further identified by GC-MS. The results demonstrated that all four tested *S. marcescens* strains could inhibit the growth of *F. oxysporum f.sp. melongenae*, with inhibition rates ranging from 93.76% to 94.02% compared to the control. These strains produced heteroauxin and its derivatives, including 3-methylindole, salicylic acid, and indole-3-acetic acid methyl ester, as confirmed by GC-MS. This study concludes that the examined *S. marcescens* strains are promising biocontrol agents and heteroauxin producers, highlighting their potential for sustainable agriculture practices.

Keywords: *Fusarium oxysporum f.sp. melongenae*; heteroauxin; *Serratia marcescens.*

1. Introduction

Various factors, including light, CO2, temperature, relative humidity, pathogens, and plant genetics, have an impact on plant growth and development (Driesen *et al.*, 2020). One of the pathogens affecting plant growth and development is the fungus *Fusarium oxysporum f.sp. melongenae*, which causes wilt disease in Solanum melongena plants (Makhlouf and Abdeen, 2015). Efforts to control wilt disease in S. melongena have traditionally involved the use of fungicides, such as daconil, topsin-M, polyram combi, captan, vitavax, dithane M-45, bavistin,

ridomil, and benlate (Ahmad *et al.*, 1996). Continuous use of fungicides leads to pathogen resistance and environmental pollution, necessitating the need for environmentally friendly plant disease control methods. One such method is the use of the rhizobacterium *Serratia marcescens*. Several researchers have reported that *S. marcescens* can control plant pathogens. Queiroz and Melo (2006) reported that *S. marcescens* strain R3.5 could control *Phytophthora parasitica*, causing gummosis disease in citrus plants. Wang *et al.* (2013) reported that *S. marcescens* strain JPP1 could control *Aspergillus parasiticus*, causing post-harvest disease in peanut plants. Purkayastha *et al.* (2018) reported that *S. marcescens* strain ETR17 could control *Lasiodiplodia theobromae, Pestalotiopsis theae, Colletotrichum camelliae,* and *Curvularia eragrostidis*, causing diseases in tea plants.

The genetic determinant influencing plant growth and development is the plant's ability to produce plant growth factors. One of the most important plant growth factors is the endogenous auxin hormone, which plays a role in cell division, cell differentiation, and fruit development (Bunsangiam *et al.*, 2021). The plant's ability to synthesize endogenous auxin hormones is influenced by limiting factors, resulting in suboptimal growth and development. Plants require the presence of exogenous auxin hormones to optimize their growth and development. One such exogenous auxin hormone is heteroauxin or indole-3-acetic acid, produced by rhizobacteria. The heteroauxin synthesized by rhizobacteria enters the plant root tissues, altering the concentration of endogenous heteroauxin in the root organ. Exogenous heteroauxin can be used to facilitate the elongation of the primary root, formation of lateral roots and root hairs (Olatunji *et al.*, 2017). The precise function of exogenous heteroauxin in relation to rhizobacteria remains uncertain; however, when rhizobacteria are in symbiotic relationship with plants in their natural habitat, the exogenous heteroauxin produced by these bacteria is capable of stimulating plant growth and development (Vocciante *et al.*, 2022). Rhizobacteria producing exogenous heteroauxin include *S. marcescens*. Several researchers have reported that *S. marcescens* produces heteroauxin and can promote plant growth. Ismawanti *et al.* (2022) reported that *S. marcescens* strain MBC1 produces heteroauxin and can improve soybean seed germination. Devi *et al.* (2016) reported that *S. marcescens* AL2-16 produces heteroauxin and can enhance the growth of *Achyranthes aspera L.* plants. Zhang *et al.* (2022) reported that *S. marcescens* PLR produces heteroauxin and can enhance the formation of lateral roots in *Arabidopsis thaliana* plants. Selvakumar *et al.* (2007) reported that *S. marcescens* strain SRM (MTCC 8708) produces heteroauxin and can enhance the growth of *Cucurbita pepo* plants. This research aims to determine the potential of *S. marcescens* as a biological agent against *F. oxysporum f.sp. melongenae* and to ascertain the potential of *S. marcescens* as a producer of heteroauxin.

2. Methodology

2.1 Research Date and Location

This research was conducted at the Plant Disease Science Laboratory of the Faculty of Agriculture, Universitas Udayana from May to August 2023. The materials used in this research include PDA media (4 grams potato extract, 20 grams glucose, 15 grams agar, Oxoid Ltd., CM139) and selective media for *S. marcescens* CHROMagar™ *Serratia*. Rhizobacteria *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, *S. marcescens* MjBL45 are isolates derived from Menjangan Island, Bali, and are among the four *S. marcescens* strains showing the best results in inhibiting the growth of *F. oxysporum f.sp. melongenae* and producing heteroauxin out of 50 tested *S. marcescens* rhizobacteria.

2.2 Antagonistic Test

The antagonistic test of *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, *S. marcescens* MjBL45 against *F. oxysporum f.sp. melongenae* using dual culture was conducted according to the Khalimi and Wirya's method (2010). A total of 10 ml of solidified PDA media in petri dishes were injected with *F. oxysporum f.sp. melongenae* in the center. *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, and *S. marcescens* MjBL45 were inoculated at four positions surrounding the fungus *F. oxysporum f.sp. melongenae* at a distance of 2 centimeters. The design used in this research was a Completely Randomized Design (CRD) with 5 treatments and 5 repetitions. The calculation of fungal colony growth rate and the percentage of bacterial inhibitory power against the fungus were based on the method established by Khalimi *et al.* (2022).

2.3 Antifungal Activity Test

Extracts of *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, and *S. marcescens* MjBL45 were obtained from dual culture of *S. marcescens* and *F. oxysporum f.sp. melongenae*. Prior to being dissolved using methanol, the colonies of *F. oxysporum f.sp. melongenae* were initially removed and the cultures of *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, *S. marcescens* MjBL45 were dissolved using methanol in a ratio of 10 *S. marcescens* culture plates in 100 ml of methanol. Subsequently, it was macerated for 48 hours, and extraction was carried out using a Vacuum Rotary Evaporator (Model HEA-02). The antifungal activity test of *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, and *S. marcescens* MjBL45 extracts against the growth of *F. oxysporum f.sp. melongenae* was conducted using the disk diffusion method.

Testing began by preparing growth media by pouring 1 ml of *F. oxysporum f.sp. melongenae* suspension onto a petri dish. Next, 10 ml of PDA media was poured into the petri dish. After the media solidified, a 6 mm diameter disk paper previously soaked in the extract of *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, and *S. marcescens* MjBL45 was inoculated. The area devoid of fungal presence is referred to as the inhibition zone. According to Paudel *et al.* (2014), the formed inhibition zone can be categorized into four categories: zone ≤10 mm as weak inhibition, zone 10-15 mm as moderate inhibition, zone 15-20 mm as strong inhibition, and zone >20 mm as very strong inhibition.

2.4 In Vitro and In Situ Salkowski Colorimetric Test

The in vitro Salkowski colorimetric test was conducted based on the method established by Gang *et al.* (2019). Cultures of *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, and *S. marcescens* MjBL45 were grown for 24 hours in Nutrient Broth medium added with 1 mM L-tryptophan. The *S. marcescens* cultures were centrifuged at a speed of 1610 x g for 30 minutes. Afterwards, the supernatant was separated from the pellet and filtered using Millipore filter paper (0.45 mm). A total of 1 ml of *S. marcescens* culture was added to a 2 ml vial and mixed with 1 ml of Salkowski reagent (1 ml of 0.5 M ferric chloride and 49 ml of 35% perchloric acid). The solution was subsequently incubated in the dark. Shall the solution in the vial turn pink, it indicates that *S. marcescens* produces heteroauxin. The in situ Salkowski colorimetric test was conducted based on the same method established by Gang *et al.* (2019). Roots of *S. melongena* plants grown in Murashige Skoog (MS) media without auxin were sterilized using 2% NaClO and rinsed with distilled water. Next, the plant roots were placed in a test tube and soaked in 10 ml of *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, and *S. marcescens* MjBL45 suspension at a population density of 106 ml-1. They were incubated for 48 hours and then transferred to a new test tube to be soaked in 10 ml of Salkowski reagent. Shall the plant roots turn pink, it indicates that heteroauxin exists in the plant roots.

2.5 GC-MS Identification of Heteroauxin Compound

Cultures of 8-hour-old *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, and *S. marcescens* MjBL45 were extracted using ethyl acetate. A total of 50 ml of culture were centrifuged at a speed of 450 rpm for 30 minutes. The supernatant was separated from the pellet and then filtered using Millipore filter paper (0.45 mm). A total of 20 ml of supernatant were acidified with 1 N HCl until reaching pH 3, followed by being partitioned with ethyl acetate three times. The ethyl acetate fraction was then separated from the water fraction. The three ethyl acetate fractions were combined into one ethyl acetate fraction and Na2SO4 was added to absorb the remaining excess water. Subsequently, Na2SO4 was filtered, and the ethyl acetate fraction was evaporated using a Vacuum Rotary Evaporator at a temperature of 40°C. The ethyl acetate fraction was subsequently dissolved in 5 ml of methanol. Identification of the heteroauxin compound was performed using Gas Chromatography-Mass Spectrometry (7890A GC-system 5975C inert XL EI/C1 MSD model G3174A, Agilent Technologies, Wilmington, DE, USA). A 2 µl sample solution of the ethyl acetate fraction was injected into GCMS. The injector temperature was kept constant at 240°C for 26 minutes. Identification of the heteroauxin compound was performed through comparison with the Willey database library. The chemical name of the analysis results adheres to the nomenclature used in the Willey database.

3. Results

3.1 Antagonistic Test

The results of the antagonistic test of *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, and *S. marcescens* MjBL45 against the fungus *F. oxysporum f.sp. melongenae* showed that each *S. marcescens* was able to inhibit the growth of the fungal colony of *F. oxysporum f.sp. melongenae* with an inhibition percentage ranging from 93.76% to 94.02% compared to the control Table 1 shows the results of the antagonistic test of S. marcescens against the fungus F. oxysporum f.sp. melongenae

Table 1. Results of the antagonistic test of *S. marcescens* against the fungus *F. oxysporum f.sp. melongenae*

Treatment	Fungal Colony Area (mm^2)	Colony Growth Rate (mm^2/day)	Inhibition Power (%)
CTRL	4112.13 a*	587.44	-
MjBL5	255.38 b	36.48	93.78
MjBL37	252.24 b	36.03	93.86
MjBL15	245.67 b	35.09	94.02
MjBL45	256.29 b	36.61	93.76

*Values in the same column followed by the same letter are not significantly different (p>0.05) according to Duncan's Multiple Range Test at the 5% level.

The fungal colony of *F. oxysporum f.sp. melongenae* in the control treatment grew properly with a colony growth rate of 587.44 mm²/day as shown in Figure 1. However, the growth of fungal colonies in the *S. marcescens* treatment was inhibited. This is evident in the low values of fungal colony area, which manifests fungal growth. The lower the value of the fungal colony area, the higher the inhibitory power of the bioagent bacteria against the fungus. Fungal growth in the *S. marcescens* treatment was inhibited, with a colony growth rate ranging from 35.09 mm²/day to 36.61 mm²/day.

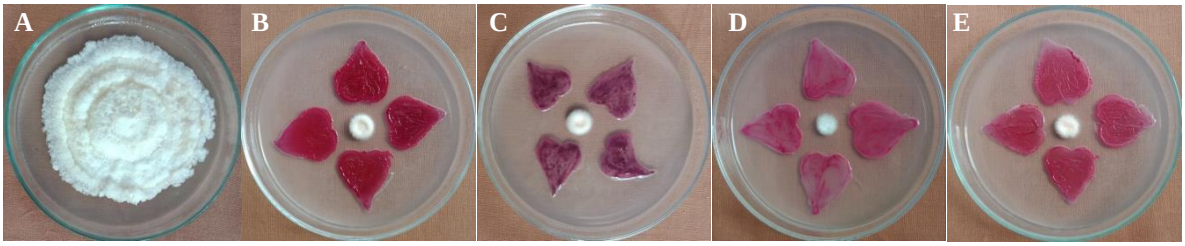

Figure 1. Results of the antagonistic test of *S. marcescens* against the fungus *F. oxysporum f.sp. melongenae*. (A). CTRL treatment, (B). MjBL5 treatment, (C). MjBL37 treatment, (D). MjBL15 treatment, and (E). MjBL45 treatment.

3.2 Antifungal Activity Test

The results of the antifungal activity test of *S. marcescens* extracts MjBL5, MjBL37, MjBL15, MjBL45 against the fungus *F. oxysporum f.sp. melongenae* showed that the extracts of *S. marcescens* MjBL5, MjBL37, MjBL15, MjBL45 possessed antifungal activity against *F. oxysporum f.sp. melongenae*, with the diameter of the inhibition zone categorized as strong inhibition, ranging between 16.23 ± 0.15 mm to 17.29 ± 0.12 mm. The extract of *S. marcescens* MjBL15 was able to inhibit the fungus *F. oxysporum f.sp. melongenae* with an inhibition zone diameter of 17.29 ± 0.12 mm while the inhibition zone diameters for the treatments with extracts of *S. marcescens* MjBL37, MjBL45, and MjBL5 were respectively 16.54 ± 0.21 mm, 16.33 ± 0.11 mm, and 16.23 ± 0.15 mm as shown in Figure 2.

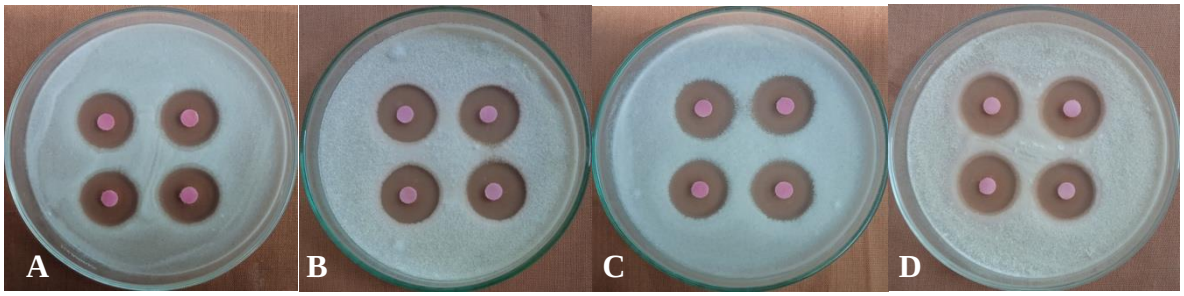

Figure 2. Results of the antifungal activity test of *S. marcescens* extract against the fungus *F. oxysporum f.sp. melongenae*. (A). Extract of *S. marcescens* MjBL5, (B). Extract of *S. marcescens* MjBL37, (C). Extract of *S. marcescens* MjBL15, (D). Extract of *S. marcescens* MjBL45.

The growth of fungi in the *S. marcescens* treatments was inhibited due to the antifungal compounds produced by *S. marcescens* through the mechanism of antibiosis. Research findings by Karthick *et al.* (2015) showed that *Serratia* sp. KC149511 produces antifungal compounds such as octadecanoic acid, phenol, 2,4-bis (1,1-dimethylethyl), and nonanoic acid-9 oxo methyl ester, which are capable of inhibiting fungi *Aspergillus flavus* and *A. niger*. Meanwhile, Wang *et al.* (2013) reported that *S. marcescens* strain JPP1 produces chitinase enzymes capable of inhibiting the fungus *A. parasiticus*.

3.3 In Vitro and In Situ Salkowski Colorimetric Test

The results of the Salkowski colorimetric test on cultures of *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, and *S. marcescens* MjBL45 showed that the four *S. marcescens* cultures changed color from clear to pink as shown in Figure 3. Salkowski reagent contains 35% perchloric acid and 10 mM ferric chloride. When reacted with *S. marcescens* cultures containing heteroauxin, a color change from clear to pink occurred. This indicates that heteroauxin is oxidized by perchloric acid, and the oxidized heteroauxin reacts with ferric chloride to form the tris-(indole-3-acetato) iron (III) complex (Kamnev *et al.*, 2001).

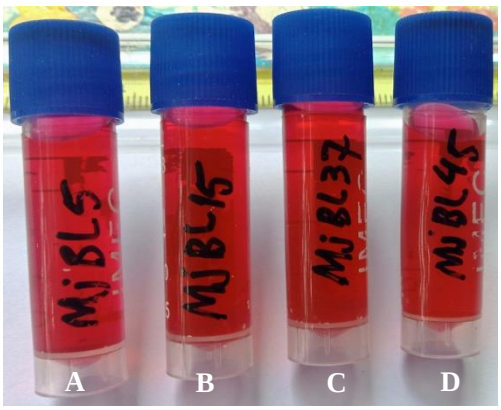

Figure 3. Results of the Salkowski colorimetric test on *S. marcescens* cultures. (A). *S. marcescens* MjBL5, (B). *S. marcescens* MjBL15, (C). *S. marcescens* MjBL37, (D). *S. marcescens* MjBL45.

The results of the Salkowski colorimetric test on Solanum melongena plant roots soaked in suspensions of *S. marcescens* MjBL5, *S. marcescens* MjBL37, *S. marcescens* MjBL15, *S. marcescens* MjBL45 showed that the plant roots changed color from white to pink. This indicated that each strain of *S. marcescens* colonized the plant roots and produced heteroauxin as shown in Figure 4. Similar research findings by Hasuty *et al.* (2018) reported that cultures of *S. marcescens subsp. marcescens* strain KB01 and *S. marcescens subsp. marcescens* strain KB05 grown in Luria Bertani broth with the addition of L-tryptophan turned pink after the addition of Salkowski reagent.

Figure 4. Results of the Salkowski colorimetric test on Solanum melongena plant roots. (A). *S. marcescens* MjBL5, (B). *S. marcescens* MjBL15, C. *S. marcescens* MjBL37, D. *S. marcescens* MjBL45.

3.4 GC-MS Identification of Heteroauxin Compound

The GC-MS analysis of the ethyl acetate fraction of *S. marcescens* MjBL5 showed that the ethyl acetate fraction of *S. marcescens* MjBL5 contained nine compounds. These compounds were 3-methylindole, 3-hydroxyindole, salicylic acid, 2.3-dihydroxy-dihydroindole, indole-3-acetaldehyde, indole-3-ethanol, heteroauxin, indole-3-acetic acid methyl ester, and indole-3-pyruvic acid as seen in Table 2.

Table 2. Chemical compounds identified in the ethyl acetate fraction of S. marcescens MjBL5

Peak	Retention Time (minutes)	Area (%)	Identified Chemical Compound	*Molecular Formula*	Molar mass ($g.mol^{-1}$)
1	16.16	2.23	3-methylindole	C_9H_9N	131.172
2	16.54	2.88	3-hydroxyindole	C_8H_7NO	133.15
3	17.23	38.34	salicylic acid	$C_7H_6O_3$	138.121
4	18.19	15.11	2.3-dihydroxy-dihydroindole	$C_8H_7NO_2$	151.16
5	18.34	4.15	indole-3- acetaldehyde	$C_{10}H_9NO$	159.18
6	19.51	3.11	indole-3-ethanol	$C_{10}H_{11}NO$	161.20
7	19.95	6.09	heteroauxin	$C_{10}H_9NO_2$	175.184
8	20.25	20.17	indole-3-acetic acid methyl ester	$C_{11}H_{11}NO$	*189.210*
9	22.57	7.91	*indole*-3-*pyruvic acid*	$C_{11}H_9NO_3$	*203.19*

The compound 3-methylindole was detected at peak 1 with a retention time of 16.16 minutes and an area percentage of 2.23%. The compound 3-hydroxyindole was detected at peak 2 with a retention time of 16.54 minutes and an area percentage of 2.88%. Salicylic acid was detected at peak 3 with a retention time of 17.23 minutes and an area percentage of 38.34%. The compound 2.3-dihydroxy-dihydroindole was detected at peak 4 with a retention time of 18.19 minutes and an area percentage of 15.11%. Indole-3-acetaldehyde was detected at peak 5 with a retention time of 18.34 minutes and an area percentage of 4.15%. Indole-3-ethanol was detected at peak 6 with a retention time of 19.51 minutes and an area percentage of 3.11%. Heteroauxin was detected at peak 7 with a retention time of 19.95 minutes and an area percentage of 6.09%. Indole-3-acetic acid methyl ester was detected at peak 8 with a retention time of 20.25 minutes and an area percentage of 20.17%. Indole-3-pyruvic acid was detected at peak 9 with a retention time of 22.57 minutes and an area percentage of 7.91%. Figure 4. shows the representative GC-MS chromatography data for the ethyl acetate fraction of *S. marcescens* MjBL5

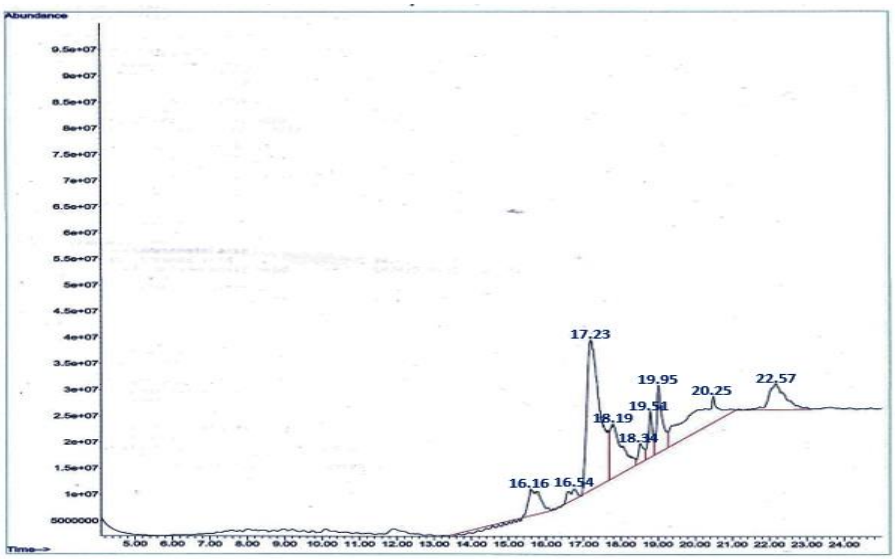

Figure 4. Representative GC-MS chromatography data for the ethyl acetate fraction of *S. marcescens* MjBL5

The GC-MS analysis of the ethyl acetate fraction of *S. marcescens* MjBL37 showed that the ethyl acetate fraction of *S. marcescens* MjBL37 contained seven compounds. These compounds were 3-methylindole, indole-3-carboxaldehyde, indole-3-acetaldehyde, indole-3-ethanol, heteroauxin, 2-hydroxyindole-3-acetic acid, and indole-3-pyruvic acid. Table 3 shows the chemical compounds identified in the ethyl acetate fraction of *S. marcescens* MjBL37.

The compound 3-methylindole was detected at peak 1 with a retention time of 12.24 minutes and an area percentage of 1.76%. The compound indole-3-carboxaldehyde was detected at peak 2 with a retention time of 16.12 minutes and an area percentage of 2.08%. The compound indole-3-acetaldehyde was detected at peak 3 with a retention time of 17.11 minutes and an area percentage of 3.80%. The compound indole-3-ethanol was detected at peak 4 with a retention time of 17.44 minutes and an area percentage of 24.86%. The compound heteroauxin

was detected at peak 5 with a retention time of 18.14 minutes and an area percentage of 7.41%. The compound 2-hydroxyindole-3-acetic acid was detected at peak 6 with a retention time of 18.21 minutes and an area percentage of 5.92%. The compound indole-3-pyruvic acid was detected at peak 7 with a retention time of 21.32 minutes and an area percentage of 54.17%. Figure 5 shows the representative GC-MS chromatography data for the ethyl acetate fraction of *S. marcescens* MjBL37

Table 3. Chemical compounds identified in the ethyl acetate fraction of *S. marcescens* MjBL37

Peak	Retention Time (minutes)	Area (%)	Identified Chemical Compound	*Molecular Formula*	Molar mass ($g.mol^{-1}$)
1	12.24	1.76	3-methylindole	*C_9H_9N*	131.172
2	16.12	2.08	indole-3-carboxaldehyde	C_9H_7NO	145.161
3	17.11	3.80	indole-3- acetaldehyde	*$C_{10}H_9NO$*	159.18
4	17.44	24.86	indole-3-ethanol	$C_{10}H_{11}NO$	161.20
5	18.14	7.41	heteroauxin	$C_{10}H_9NO_2$	175.184
6	18.21	5.92	2-hydroxyindole-3-acetic acid	*$C_{10}H_9NO_3$*	191.18
7	21.32	54.17	*indole-3-pyruvic acid*	$C_{11}H_9NO_3$	*203.19*

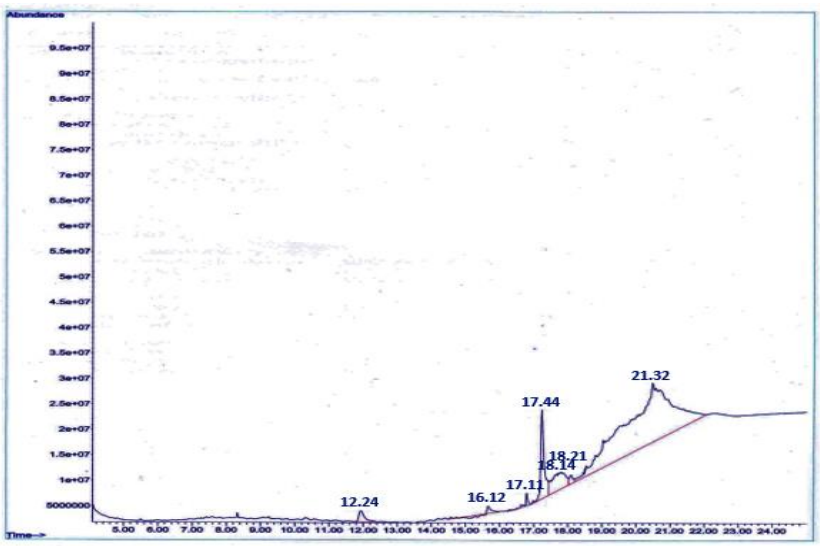

Figure 5. Representative GC-MS chromatography data for the ethyl acetate fraction of *S. marcescens* MjBL37

The GC-MS analysis of the ethyl acetate fraction of *S. marcescens* MjBL15 showed that the ethyl acetate fraction of *S. marcescens* MjBL15 contained six compounds. These compounds were 3-methylindole, indole-3-carboxaldehyde, 3-methyloxindole, indole-3-acetaldehyde, heteroauxin, and indole-3-pyruvic acid. Table 4 shows the chemical compounds identified in the ethyl acetate fraction of *S. marcescens* MjBL15.

Table 4**.** Chemical compounds identified in the ethyl acetate fraction of *S. marcescens* MjBL15

Peak	Retention Time (minutes)	Area (%)	Identified Chemical Compound	*Molecular Formula*	Molar mass ($g.mol^{-1}$)
1	12.15	8.16	3-methylindole	*C_9H_9N*	131.172
2	17.25	20.69	indole-3-carboxaldehyde	C_9H_7NO	145.161
3	18.55	8.47	3-methyloxindole	C_9H_9NO	*147.17*
4	18.77	3.32	indole-3- acetaldehyde	*$C_{10}H_9NO$*	159.18
5	20.35	21.44	heteroauxin	$C_{10}H_9NO_2$	175.184
6	21.67	37.92	*indole-3-pyruvic acid*	$C_{11}H_9NO_3$	*203.19*

The compound 3-methylindole was detected at peak 1 with a retention time of 12.15 minutes and an area percentage of 8.16%. The compound indole-3-carboxaldehyde was detected at peak 2 with a retention time of 17.25 minutes and an area percentage of 20.69%. The compound 3-methyloxindole was detected at peak 3 with a retention time of 18.55 minutes and an area percentage of 8.47%. The compound indole-3-acetaldehyde was detected at peak 4 with a retention time of 18.77 minutes and an area percentage of 3.32%. The compound heteroauxin was detected at peak 5 with a retention time of 20.35 minutes and an area percentage of 21.44%. The compound indole-3-pyruvic acid was detected at peak 6 with a retention time of 21.67 minutes and an area percentage of 37.92%. Figure 6 shows the representative GC-MS chromatography data for the ethyl acetate fraction of S. marcescens MjBL15

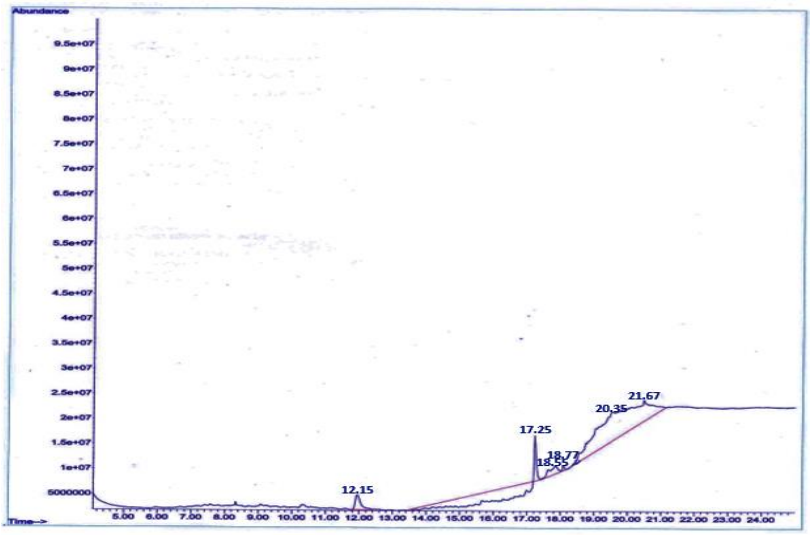

Figure 6. Representative GC-MS chromatography data for the ethyl acetate fraction of *S. marcescens* MjBL15

The GC-MS analysis of the ethyl acetate fraction of *S. marcescens* MjBL45 showed that it contained eight compounds. These compounds were 3-methylindole, indoline-3-ol, indole-3-carboxaldehyde, indole-3-acetaldehyde, indoline-3-carboxylic acid, heteroauxin, indole-3-acetic acid methyl ester, and indole-3-pyruvic acid. Table 5 shows the chemical compounds identified in the ethyl acetate fraction of *S. marcescens* MjBL45

Table 5. Chemical compounds identified in the ethyl acetate fraction of *S. marcescens* MjBL45

Peak	**Retention Time (minutes)**	**Area (%)**	**Identified Chemical Compound**	***Molecular Formula***	**Molar mass ($g.mol^{-1}$)**
1	12.25	2.62	3-methylindole	C_9H_9N	131.172
2	15.95	2.12	indoline-3-ol	C_8H_7NO	135.16
3	17.35	21.90	indole-3-carboxaldehyde	C_9H_7NO	145.161
4	18.25	4.92	indole-3- acetaldehyde	$C_{10}H_9NO$	159.18
5	19.12	6.83	indoline-3-carboxylic acid	C_9H_7NO	161.16
6	19.76	10.41	heteroauxin	$C_{10}H_9NO_2$	175.184
7	20.52	18.14	indole-3-acetic acid methyl ester	$C_{11}H_{11}NO$	*189.210*
8	23.12	33.06	*indole-3-pyruvic acid*	$C_{11}H_9NO_3$	*203.19*

The compound 3-methylindole was detected at peak 1 with a retention time of 12.25 minutes and an area percentage of 2.62%. The compound indoline-3-ol was detected at peak 2 with a retention time of 15.95 minutes and an area percentage of 2.12%. The compound indole-3-carboxaldehyde was detected at peak 3 with a retention time of 17.35 minutes and an area percentage of 21.90%. The compound indole-3-acetaldehyde was detected at peak 4 with a retention time of 18.25 minutes and an area percentage of 4.92%. The compound indoline-3-carboxylic acid was detected at peak 5 with a retention time of 19.12 minutes and an area percentage of 6.83%. The compound heteroauxin was detected at peak 6 with a retention time of 19.76 minutes and an area percentage of 10.41%. The compound indole-3-acetic acid methyl ester was detected at peak 7 with a retention time of 20.52

minutes and an area percentage of 18.14%. The compound indole-3-pyruvic acid was detected at peak 8 with a retention time of 23.12 minutes and an area percentage of 33.06%. Figure 7 shows the representative GC-MS chromatography data for the ethyl acetate fraction of S. marcescens MjBL45.

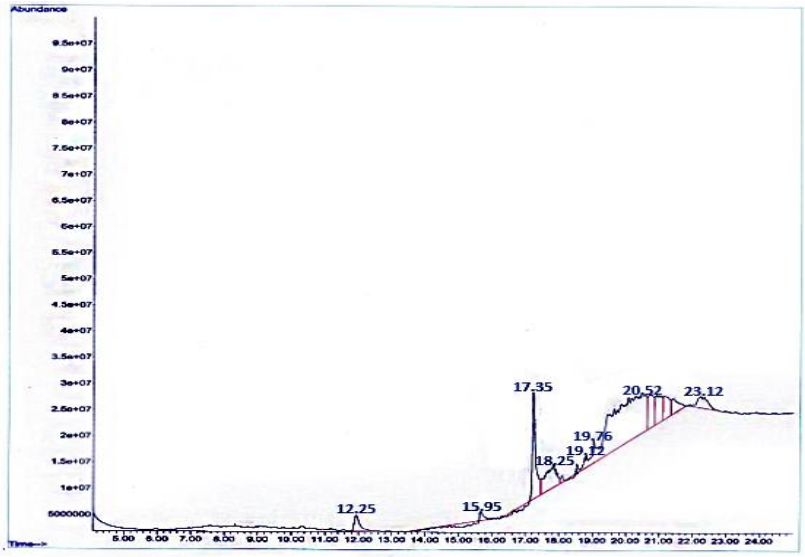

Figure 7. Representative GC-MS chromatography data for the ethyl acetate fraction of *S. marcescens* MjBL45

The heteroauxin compounds produced by *S. marcescens* in plant roots contributed to the regulation of plant physiological processes. Heteroauxin's role in regulating plant physiological processes referred to the acid growth hypothesis and the gene activation hypothesis. According to the acid growth hypothesis, heteroauxin was bound by the receptor protein ABP1, which interacted with the CBP1 protein located in the plasma membrane. The complex bond of heteroauxin and proteins ABP1 and CBP1 activated the ATPase proton pump. The ATPase proton pump, located in the plant cell membrane, functioned to expel H+ from the cytoplasm to the cell wall. Heteroauxin enhanced the activity of the ATPase proton pump, leading to a significant expulsion of H+ from the cytoplasm to the cell wall. The increased concentration of H+ caused the cell wall pH to become more acidic. The acidic condition of the cell wall activated the enzymes expansin and xyloglucan endotransglycosylase. These enzymes loosened the cell wall by breaking the hydrogen bonds in the cross-linking of microfibrils (cellulose, hemicellulose, and pectin fibers). The disrupted cross-links resulted in a more relaxed and softer cell wall. Activation of ATPase can also induce hyperpolarization of the plasma membrane and activate K+ in the plasma membrane channels. This led to the influx of ions into the cell, increasing the ion concentration inside the cell. The increased ion concentration affected water entry into the cell through osmosis. The influx of water into the cell caused cell turgor pressure to increase. The increased pressure, supported by the relaxed cell wall, resulted in cell elongation and enlargement (Rechenmann, 2010). Meanwhile, according to the gene activation hypothesis, heteroauxin was bound by the transport inhibitor response 1 (TIR1) protein located in the nucleus. TIR1 bound with cullin protein, S-phase kinase protein, and RBX1 protein to form the ubiquitin SCF TIR1 complex and bound with the Aux/IAA inhibitor protein located in the auxin-activated transcription factor (ARFs). The auxin-activated transcription factor moved to the auxin-dependent promoter to initiate the transcription of target genes or auxin-regulated genes with the assistance of the ubiquitin SCF TIR1 complex (Chapman and Estelle, 2009; Abel and Theologis, 2010).

4. Discussion

The in situ Salkowski colorimetric test results indicated that *S. marcescens* colonized the roots of S. melongena plants. The rhizobacterium *S. marcescens* converted the amino acid tryptophan contained in plant root exudated into heteroauxin compounds and heteroauxin derivatives, including 3-methylindole, 3-hydroxyindole, 3-methyloxindole, salicylic acid, 2.3-dihydroxy-dihydroindole, indoline-3-ol, indole-3-carboxaldehyde, indole-3-acetaldehyde, indole-3-ethanol, 2-hydroxyindole-3-acetic acid, indoline-3-carboxylic acid, indole-3-acetic acid methyl ester, and indole-3-pyruvic acid. Arora *et al.* (2015) reported that *Pseudomonas sp. bacteria* degraded heteroauxin into 3-methylindole, which subsequently became salicylic acid and catechol. Lebuhn *et al.* (1997) reported that the compound indole-3-ethanol is an inactive heteroauxin. If the plant requires heteroauxin, the enzyme indole-3-ethanol oxidase occurs in the plant. It converts indole-3-ethanol into active heteroauxin with the help of oxygen. Oberhansli *et al.* (1991) reported that tryptophan is converted by bacteria into indole-3-pyruvic

acid, which then can be converted into heteroauxin and indole-3-aldehyde. Kumavath *et al.* (2017) reported that indole-3-pyruvic acid spontaneously converts into indole-3-aldehyde. Nakajima *et al.* (2002) reported that indole-3-aldehyde has heteroauxin-like activity.

The heteroauxin compounds and their derivatives produced by *S. marcescens* helped plants in achieving a state of heteroauxin homeostasis, an internal equilibrium within the plant's bodily system that functions and interacts appropriately to meet the needs of heteroauxin in cells, tissues, and organs of the plant. The concentration of heteroauxin within plant cells was regulated through mechanisms of heteroauxin biosynthesis, heteroauxin transport, inactivation of heteroauxin, degradation of inactive heteroauxin, signal transduction, and the conjugation or deconjugation of inactive heteroauxin from methyl esters, sugars, and amino acids (Woodward and Bartel, 2005). Heteroauxin transport within plant cells occurred in the form of indole-3-acetic acid methyl ester. The compound indole-3-acetic acid methyl ester was nonpolar, allowing it to diffuse easily across plant cell membranes and be transferred from one cell to another. If the plant requires heteroauxin, the indole-3-acetic acid methyl ester is hydrolyzed and reactivated into heteroauxin by plant esterase enzymes (Yang *et al.*, 2008). Qin *et al.* (2005) reported that the enzyme carboxyl methyltransferase in Arabidopsis thaliana can methylate heteroauxin compounds to indole-3-acetic acid methyl ester. Muller (2011) reported that indole-3-acetic acid methyl ester is an inactive heteroauxin conjugated with an ester compound.

5. Conclusions

The research results indicated that the four tested *S. marcescens* strains had potential as biological agents due to their ability to inhibit the growth of *F. oxysporum f.sp. melongenae*, with inhibition rates ranging from 93.76% to 94.02% compared to the control. Furthermore, the *S. marcescens* extract exhibited antifungal activity against *F. oxysporum f.sp. melongenae*, with the diameter of the inhibition zone being categorized as strong inhibition. The four tested *S. marcescens* strains produced heteroauxin compounds as indicated by in vitro and in situ Salkowski colorimetric tests. Based on the GC-MS analysis, they produced heteroauxin derivatives, including 3-methylindole, 3-hydroxyindole, 3-methyloxindole, salicylic acid, 2.3-dihydroxy-dihydroindole, indoline-3-ol, indole-3-carboxaldehyde, indole-3-acetaldehyde, indole-3-ethanol, 2-hydroxyindole-3-acetic acid, indoline-3-carboxylic acid, indole-3-acetic acid methyl ester, and indole-3-pyruvic acid.

Author Contributions

Conceptualization, I. B. G. P. and K.K.; methodology, K.K.; software, I. B. G. P.; validation, I. B. G. P., K.K. and C.B. B.; formal analysis, C. B. B.; investigation, K. K.; resources, I. B. G. P.; data curation, C. B. B.; writing—original draft preparation, K. K.; writing—review and editing, K. K.; visualization, I. B. G. P.; supervision, I. B. G. P.; project administration, K. K.; funding acquisition, K.K. All authors have read and agreed to the published version of the manuscript.

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The authors declare there no conflict of interest

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