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Characterization of Promising Antifungal Bioactive Compounds from Endophytic Actinobacterium, *Streptomyces rochei* OM182844

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ABSTRACT

This study evaluated the activity of a newly identified endophytic actinobacterium strain *Streptomyces rochei* OM182844 as an antifungal agent against soil-borne pathogenic fungi, *Fusarium oxysporum* f.sp. *cucumerinum*, *Sclerotinia rolfsii*, and *Pythium aphanidermatum*. The antifungal activity was performed using *Strep. rochei* culture and its crude culture filtrate (CCF), and they showed superiority in the antagonistic activity towards the three tested pathogens as inhibition of growth on plates and reduction of mycelial growth weight in broth. *Strep. rochei* was a high producer of cell-wall degrading enzymes (chitinases and cellulases) as a tool used to degrade pathogenic fungal-cell walls. In addition, the released volatile organic compounds (VOCs) were submitted for GC/MS analysis; it showed that the most abundant compounds were Hexadecanoic acid, trimethylsilyl ester (CAS) followed by iridomyrmecin which have antifungal activities. Hence, the success of our idea of using actinobacteria isolated from healthy plants as a biocontrol agent is a promising point for much research towards the production of unconventional and environmentally safe biocides.

INTRODUCTION

Recent technical advancements have demonstrated the value of microbial inoculants, especially endophytic microorganisms, as chemical substitutes for encouraging environmentally friendly, sustainable farming techniques. Endophytic microorganisms have several characteristics, the most important of which is the secretion of multifunctional bioactive substances that promote plant growth in addition to the ability to inhibit plant pathogens, which ultimately leads to a reduction in the use of chemical fertilizers and pesticides. Actinobacteria produce 10.000 of the 23.000 bioactive compounds produced by microbes, accounting for 45% of all bioactive microbial metabolites known (Janatiningrum *et al.*, 2022). The endophytic actinobacteria, especially those belonging to the genus *Streptomyces* sp., colonize plant tissues efficiently, from roots to aerial sections, in addition to being an active component that

makes up a large proportion of the soil microbiome.

The endophytic actinobacteria can control the physiological processes in their host plants by inducing resistance to environmental and biological stress, in addition to their ability to secrete unique bioactive natural compounds that may have beneficial uses in biological pests' control (Mitra *et al*., 2022).

Endophytic *Streptomyces* sp. is considered one of the most essential microorganisms in agriculture because it improves crop output by stimulating plant growth and immunological response, reducing plant diseases via competition, and actively participating in metabolisms. On the other hand, endophytic actinomycetes' synthesis of antibiotics, siderophores, and antioxidants is a reliable source of biotechnologically beneficial bioactive compounds and secondary metabolites (Singh *et al.*, 2022). They can also produce extracellular cell wall-degrading enzymes (CWDEs), such as chitinase, β-1,3-glucanase, and cellulase, which are used either directly or indirectly in the biological control of soil-borne fungi because chitin is the main component of the fungal cell, or indirectly through the use of purified protein or gene manipulation (Ruangwong *et al*., 2022).

Volatile organic compounds VOCs are low molecular weight molecules that quickly evaporate at room temperature and atmospheric pressure, allowing them to diffuse through the atmosphere and soil (Tilocca *et al*., 2020). Actinobacteria have been found to decrease the development of fungal, and bacterial infections by releasing VOCs, induce systemic resistance in crops as well, as the ability to enhance plant growth (Ayed *et al*., 2021). *Streptomyces* is an excellent reservoir for novel natural beneficial metabolites that can protect plant hosts from infection pathogens, including the VOCs, that protect plants directly or indirectly against phytopathogens through fumigation. These VOCs play a vital role in soil microbial relationships, especially antagonism and parasitism (de Brito *et al*., 2022).

Hence, a series of laboratory experiments were designed to evaluate endophytic *Streptomyces rochei* as biocontrol agents against phytopathogenic fungi through their ability to produce extracellular hydrolytic enzymes and other biocontrol compounds such as volatile organic compounds (VOCs), which can inhibit the growth of plant pathogens and thus act as biocontrol agents.

MATERIALS AND METHODS

Source of Actinobacteria:

Streptomyces rochei OM182844, a newly endophytic actinobacteria (EA) strain isolated from healthy cucumber plants and identified (El-Akshar *et al.,* 2022) was used in the current study. EA strain was grown on the International *Streptomyces* (ISP-4) medium (Shirling and Gottlieb, 1966) containing (gL^{-1}): 10.0 soluble starch, 1.0 $MgSO_4$, 1.0 K_2HPO_4, 2.0 $(NH_4)_2SO_4$, 2.0 $CaCO_3$, 1.0 NaCl, 0.001 $MnCl_2$, 0.001 $FeSO_4$, 0.001 $ZnSO_4$. $7H_2O$, 20.0 agar, pH 7.5 ± 0.2 at 35±2°C for 7 days, then kept at 4°C for all subsequent experiments.

Source of Pathogenic Fungi:

Three pathogenic fungi, *Fusarium oxysporum* f.sp. *cucumerinum*, *Sclerotinia rolfsii*, and *Pythium aphanidermatum*, were obtained from the Plant Pathology Institute, Agricultural Research Center, Giza, Egypt. Fungal strains were grown on potato dextrose agar (PDA) medium containing (gL^{-1}): 200.0 infused potatoes, 20.0 dextrose, 20.0 agar, for 4 days at 28±2°C, then kept at 4°C.

Antifungal Activity of *Strep. Rochei*:

Dual Culture Assay (opposite growth):

Strep. rochei was tested for its capacity to suppress three pathogenic fungi separately using a dual-culture experiment. Firstly, 7 days-old *Strep. rochei* was streaked on one side of a Petri plate (90 mm) containing a mixture of International Streptomyces Project (ISP-4) (Shirling and Gottlieb, 1966) and PDA media and incubated at 35±2°C for 2 days before the fungal strain was inoculated in the center of the plate. Plates containing only PDA medium and inoculated with each fungus separately were used as control. All plates were re-incubated

for 7 days at 28±2°C. Finally, the radial mycelium of each fungus towards and away from the actinobacteria was measured and inhibition of fungal growth was calculated as a percentage using the following formula by (Rahman *et al*., 2009):

$$\text{Growth inhibition (\%)} = \frac{R - r}{R} \times 100$$

Where, R: Radius of the fungal growth in the control plate, r: Radius of the fungal growth in the test plate.

Inverted Bioassay (inverted plates):

The inhibition of fungal mycelial growth by the secreted volatiles by *Strep. rochei* was assayed on an inverted plate according to (Garbeva *et al*., 2014). 7 days-old *Strep. rochei* was inoculated at the center of a one-half Petri dish containing ISP-4 medium, while each pathogenic fungus (4 days old) was inoculated onto a half of another Petri dish containing PDA. Then, both halves were placed face to face, closed and wrapped with parafilm to prevent any losses of volatiles formed, and incubated at 30±2°C for 14 days to allow fungi to exposure to released volatiles. For control plates, half of a plate was inoculated with each fungus, and the other half was filled with ISP-4 medium and left un-inoculated and closed in the same manner. All plates were observed for the fungal growth and measured for growth inhibition (%) calculation using the abovementioned formula.

Reduction of Fungal Biomass by Crude Culture Filtrate (CCF):

Strep. rochei was grown on ISP-4 broth medium and incubated at 35±2°C for 7 days under shaking (200 rpm), then centrifuged (centrifuge Tube GKF, China) at 10,000 rpm for 20 min to get the crude culture filtrate (CCF). At the same time, each pathogenic fungus was grown on PDA medium for 4 days at 28±2°C, then 0.5 cm discs were prepared. 10 ml of each CCF was transferred to a 100 ml Erlenmeyer flask containing 40 ml of potato dextrose broth medium, mixed well, and carefully inoculated with a 0.5 cm disc of each pathogenic fungus separately. Flasks amended with 10 ml of sterilized distilled water instead of CCF served as a control. All flasks were incubated at 28±2°C, and the fungal mats were harvested after 3 and 7 days, then dried at 70°C to a constant weight. The reduction (%) in pathogenic fungal growth was calculated using the following equation (El-Boghdady 1993):

$$\text{Reduction of fungal biomass (\%)} = \frac{C - T}{C} \times 100$$

Where, C: fungal mycelium dry weight in control flask,

T: fungal mycelium dry weight in treatment flask.

Cell-Wall Degrading Enzymes:

Chitinase Activity (EC 3.2.1.14):

Strep. rochei was cultured on colloidal chitin broth medium containing (gL^{-1}): 6.0 Na_2HPO_4, 3.0 KH_2PO_4, 1.0 NH_4Cl, 0.5 NaCl, 0.05 yeast extract, 15.0 agar and 1% (w/v) colloidal chitin) and incubated at 35±2°C under shaking (150 rpm). After 7 days, the cultures were filtered by Whatman No.1 filter paper. Then the filtrates were centrifuged at 8,000 rpm for 20 min. After that, 1.0 ml of 1.0% colloidal chitin prepared in 0.05 M citrate phosphate buffer (pH 6.6) was transferred to a sterilized test tube, and 1.0 ml of the *Strep. rochei* supernatant was added, then the mixture was incubated under shaking (180 rpm) for 60 min at 37°C. The reaction was stopped by immersing the tubes in boiling water for 5 min and then allowing them to cool to room temperature. The released reducing sugar N-acetylglucosamine (NAG) was estimated using 3,5-dinitrosalicylic acid (DNS) reagent. A spectrophotometer (Sco. Tech, SP UV-19) was used to detect the produced orange color at 540 nm. Chitinase activity was measured in units (U), which is the quantity of crude enzyme solution required to catalyze the release of 1.0 mol of NAG/h/37°C (Sadeghi *et al.* 2012).

Cellulase Activity (EC 3.2.1.4):

Strep. rochei was cultured in carboxy methyl cellulose (CMC) broth medium containing (gL^{-1}), (1.0 $NH_4H_2PO_4$, 0.2 KCl, 1.0 $MgSO_4.7H_2O$, 1.0 Yeast extract, 26.0 CMC) (Neethu *et al.*, 2012), and shaking

incubated at 35ºC and 150 rpm for 4 days. The supernatant (crude enzyme) was obtained after centrifugation at 10,000 rpm for 10 min under cooling (4ºC). Cellulase activity (CMCase) was quantified using the most common method by a DNS (3.5-dinitrosalicylic acid) spectrophotometric assay (Miler, 1959) as follows: mixing 300 µl of cell-free supernatant with 900 µl of 1.0% CMC in 0.05 M sodium acetate buffer (pH 7). The reaction mixture was incubated at 50°C for 25 min; then, 1800 µl DNS reagent (5.0 g DNS was dissolved in 250 mL of distilled water at 80°C) was added and boiled for 5 min. After that, the samples were left to cool down for 5 min before the absorbance was recorded at 550 nm using a spectrophotometer. Briefly, one unit (U/mL) of enzyme activity is defined as the amount of enzyme required to liberate 1 µmol of reducing sugar (D-glucose) from CMC per min under the assay conditions.

GC-Mass Analysis of The Produced Secondary Volatile Metabolites:

Firstly, *Strep. rochei* was cultured in 250 mL Erlenmeyer flasks containing 100 mL of ISP-4 medium at 35°C for 7 days under shaking (150 rpm). The secreted metabolites were extracted as described by Abdel-Aziz *et al.* (2021) as follows: 100mL ethyl acetate was added to 100mL of culture medium, and the mixture was sonicated for 10 min to disintegrate cells, and then the mixtures were centrifuged at 10,000 rpm for 20 min. The ethyl acetate phase was separated from the cell debris, and the aqueous phase was discarded while the solvent fraction was separated by a separating funnel and stored at -20°C till use. Briefly, one µL of each crude ethyl acetate extract sample was injected into the GC-MS instructor (Agilent Technology 7890B gas chromatograph with a split detector and a mass spectrometer detector (5977A). The carrier gas was hydrogen and applied at a constant flow rate of 1.0 mL/min. Temperatures were set at 250, 280 and 260°C for the injector, the detector, and the oven, respectively. The initial identification of the detected compounds was performed based on the comparison of their relative retention times and mass spectra with those of the NIST and WILLY library data of the GC/MS system. Then, the percent relative peak area was used to calculate the quantity of these compounds. This analysis was achieved at Central Laboratories Network, National Research Centre, Dokki, Cairo, Egypt (https://www.nrc.sci.eg/centrel-labs/) using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column.

RESULTS AND DISCUSSION

Suppression of Pathogenic Fungal Growth by *Strep. Rochei*:

Dual Culture Assay (opposite growth):

Strep. rochei strongly inhibited the mycelial growth of the three pathogenic fungi under study after 3 and 7 days. Figure 1(a) showed that *Strep. rochei* was more effective in inhibiting the growth of both *P. aphanidermatum* and *F. oxysporum* than *S. rolfsii* and the inhibition percentages of their growth ranged from (84.43-90%), (85.33-87%), (68.22-74.33%), respectively. Although no direct contact occurred between *Streptomyces* and fungi, the fungal growth was suppressed, suggesting the secretion of some diffusible inhibitory substances in the medium that affected the pathogenic fungal mycelia, as shown in Figure 2 (A-F). Furthermore, it was observed that all pathogenic fungi were inhibited after three days at a higher rate than after seven days. This notice was realistic because the first three days of the fungal life cycle is the stage of the rapid growth of the mycelium, and then it tends to form spores and other structures, and therefore the inhibition of its growth takes place during the first three days of its life cycle (Lee *et al*., 2021).

Different actions used by the endophytic actinomycetes to control plant fungal diseases were represented by Al-Raish *et al*. (2020), including antibiotics, secretion of degrading enzymes such as chitinases, β-1,3-glucanases, and proteases, along with the production of enzyme inhibitors and signaling molecules. Additionally, Corral *et al.,* (2022) attributed the ability of *Streptomyces* to reduce the growth of pathogenic fungi to the

production of antifungal compounds through their additive or synergistic effects on these fungi. The study findings using, *Strep. rochei* strain SM3 that suppressed the growth of *S. sclerotiorum* and its resulting disease progression was consistent with (Gebily *et al.*, 2021) report. Kanini *et al*. (2013) found that 39 out of 528 *Streptomyces* isolates appeared their antifungal activity against the growth of *F. oxysporum* f.sp. *lycopersici*, among them, the highest active one was identified as *Strep. rochei*.

Inverted Bioassay (face to face growth):

The inhibitory effect of VOCs secreted by *Strep. rochei* against *P. aphanidermatum*, *F. oxysporum* and *S. rolfsii* after 3 and 7 days was clear as shown in (Figure 1b). It was noted that the period after 3 days was higher in inhibition than 7 days; this is expected and accurate. In addition, results showed that *Strep. rochei* was more efficient at reducing the radial growth of *P. aphanidermatum* than the other two pathogenic fungi. In control, all tested fungi grew above the medium at a regular growth rate, confirming our theory that VOCs released by *Strep. rochei* were responsible for this inhibition. The produced VOCs by microorganisms are usually secreted in the form of complex mixtures of low molecular weight lipophilic compounds that could be used as pesticide compounds (Gu *et al*., 2007). In confirmation of this, it was found that several phytopathogens such as *Penicillium italicum, Botrytis cinerea, Rhizoctonia solani, F. moniliforme, F. oxysporum* and *S. sclerotiorum* have successfully been suppressed by VOCs from *Streptomyces* strains (Yang *et al*., 2019). Moreover, the VOCs of *Streptomyces* strains reduced both the aerobic and mycelium fungi growing inside the medium by (22.5 to 96.7%) and (0.0 - 9.4%), respectively (Corral *et al*., 2022).

Reduction of Fungal Biomass by Crude Culture Filtrate (CCF):

Crude culture filter (CCF) of *Strep. rochei* was evaluated for its suppressive effect on the growth of pathogenic fungi after 3 and 7 days. The growth weight of all pathogenic fungi was affected by the presence of *Strep. rochei* CCF (Fig. 3). It was clear that the maximum reduction of three fungal growth was observed after 3 days with CCF. Additionally, the CCF of *Strep. rochei* showed the highest reduction activity against *F. oxysporum* after 3 and 7 days compared to the other pathogenic fungi, which recorded 73.29 and 76.95% after 3 and 7 days, respectively (Fig. 3 b). On the other hand, *S. rolfsii* was the most tolerant pathogen for CCF of *Strep. rochei* and showed the lowest reduction ratio in its growth (67.96-58.5%) after 3 and 7 days, respectively (Fig. 3 c).

The toxicity of CCF of *Strep. rochei* might be attributed to the production of secondary metabolites such as antibiotics, siderophores, and hydrolytic enzymes in the culture media. This illation was confirmed by Alblooshi *et al*. (2022), who revealed that the culture filtrate of *Strep. coeruleoprunus* was effective in inhibiting the growth of *F. oxysporum* and decreasing its dry weight. Also, they did not observe any colonies or mycelial growth of *F. oxysporum* when the concentration of culture filtrate of *Strep. coeruleoprunus* reached 100%. Their results confirmed that the antifungal action of endophytic *Streptomyces* species might be associated with the production of cell-wall degrading enzymes and the production of volatiles. Similar results were observed that the cell-free supernatant of *Streptomyces* sp. can effectively inhibit the mycelial growth of many phytopathogenic fungi (Ruangwong *et al.*, 2022).

Cell Wall Degrading Enzymes (CWDEs) Produced by *Strep. Rochei*:

Based on the results, *Strep. rochei* was able to produce two cell wall-degrading enzymes (CWDEs) that limit the growth of target fungi. *Strep. rochei* synthesis 10.78 U/mL of cellulase enzyme, while chitinase activity was recorded at 11.57 mM N-acetyl glucosamine/1.0mL/1h (Fig. 4a). According to our results, the growth inhibition of the pathogenic fungi under study may be due to the secretion of these cell wall-degrading enzymes. Where cellulose accounts for about 20% of the cell wall composition in *P.*

aphanidermatum, while chitin accounts for a large percentage of cell wall composition in both *F. oxysporum* and *S. rolfsii*. (Schoffelmeer *et al*., 1999). Hence, the chitinases and cellulases produced by the two *Strep. rochei* are responsible for the degradation of the cell wall and, thus, the inhibition of their growth.

Previous research demonstrated that endophytic actinobacteria had produced enzyme inhibitors, signaling molecules, and/or immunomodulators in addition to antibiosis and the secretion of lytic enzymes such chitinases, -1,3-glucanases, proteases, and lipases to manage plant fungal infections (Al Raish *et al.*, 2020). Additionally, the creation of antifungal metabolites results in hyphal growth and distortion, which hinder spore germination and cause cell lysis (Liu *et al.*, 2019).

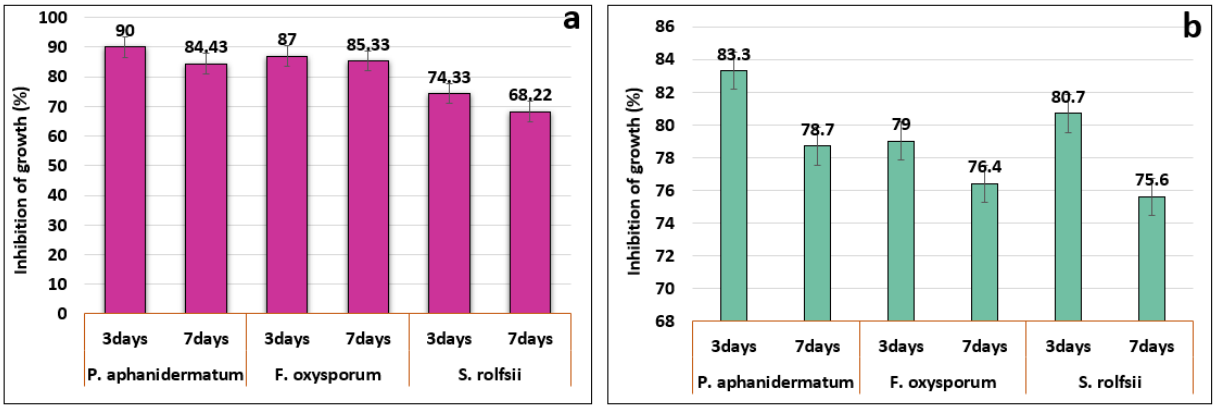

Fig. 1. Inhibition percentage of three pathogenic fungi growth (*P. aphanidermatum, F. oxysporum* and *S. rolfsii*) as affected by endophytic *Strept. rochei*; **a)** dual culture assay (opposite growth); **b)** volatile compounds (inverted plates).

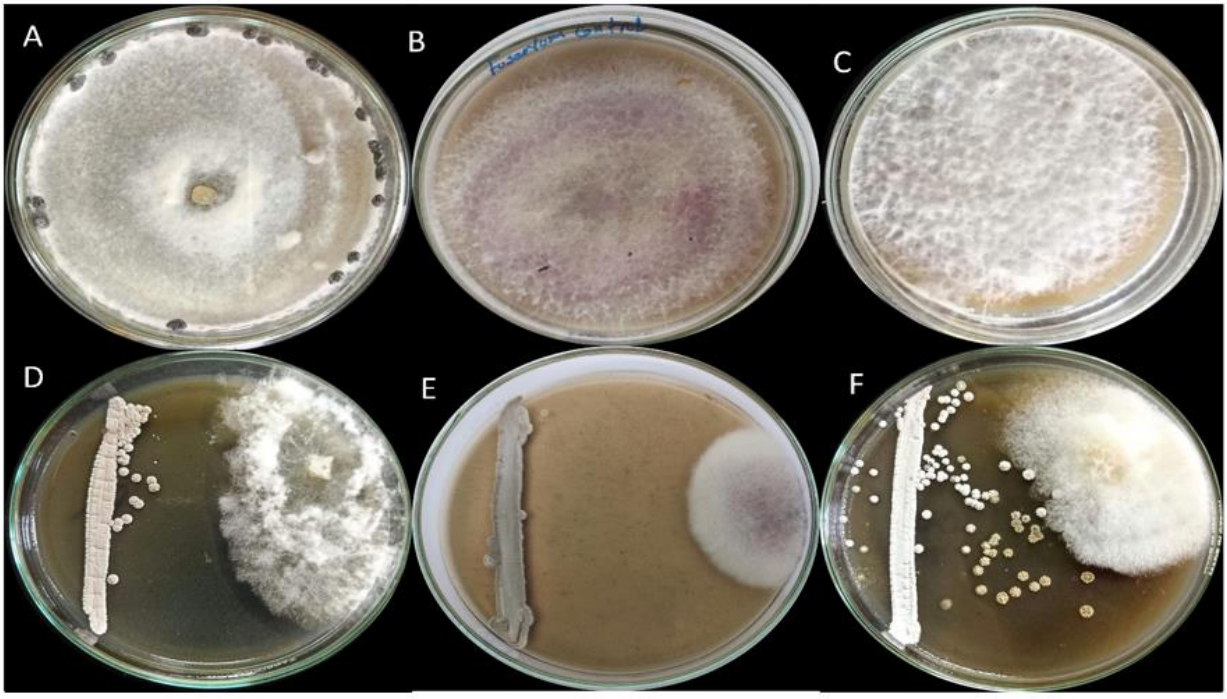

Fig. 2. **A)** *S. rolfsii,* **B)** *F. oxysporum* **C)** *P. aphanidermatum,* **D-F)** antagonistic activity of *Strep. rochei* against three pathogenic fungi.

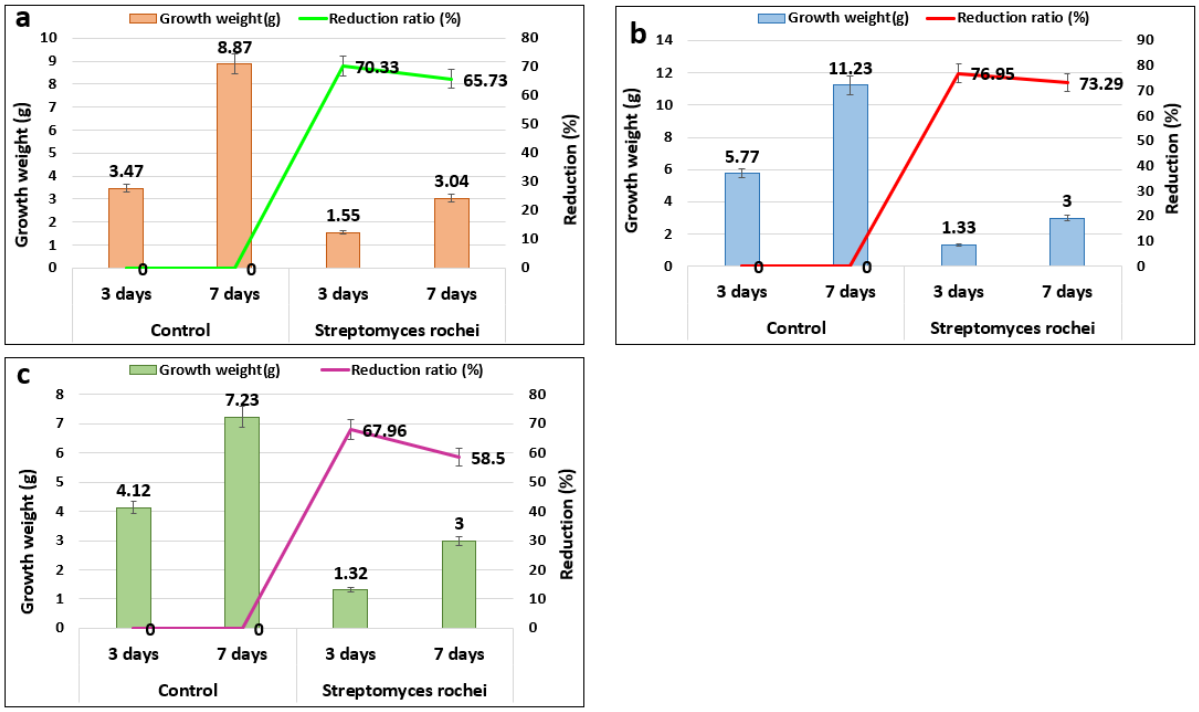

Fig. 3. Impacts of CCF of endophytic *Strept. rochei* on fungal growth weight (g); reduction (%) of **a)** *P. aphanidermatum,* **b)** *F. oxysporum,* **c)** *S. rolfsii*.

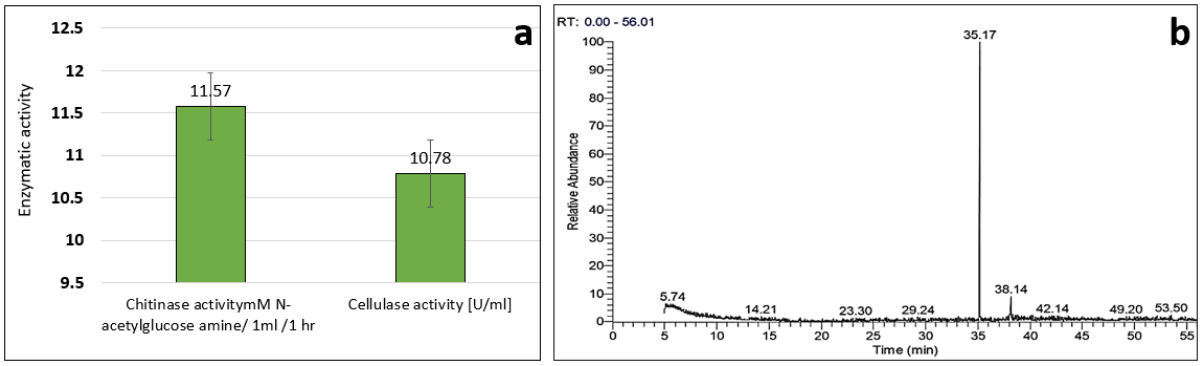

Fig. 4. a) Quantitative production of two cell-wall degrading enzymes by *Strept. rochei,* **b)** GC–MS profile of volatile compounds secreted by *Strep. rochei*

GC/MS Profile of VOCs Produced by *Strep. Rochei*:

Analyses of VOCs produced by *Strep. rochei* was done by GC-MS and their compositions are displayed in Figure 4b and Table 1. *Strep. rochei* produced a high number of metabolites that have values of biological activities. GC-MS analysis revealed that 12 out of 26 compounds in the ethyl acetate extracts of VOCs secreted by *Streptomyces rochei* were identified as antifungal **(**Table 1). Among the VOCs detected, the most abundant compound in the cell-free filtrate of *Strep. rochei* was Hexadecanoic acid, trimethylsilyl ester (CAS), that yielded at a rate of 55.92% and was found to be an antifungal, antimicrobial, and antigenotoxicity compound (Saddiq *et al.*, 2022). In addition, iridomyrmecin was found in considerable amounts, and it was found to be a fungicide (Karsli and shain 2021). Furthermore, Jasmonic acid, known as an antifungal compound (Lee *et al*., 2022), was also detected in *Strep. rochei* metabolites (Table 1).

Table 1. GC-MS profile of secondary metabolites secreted by *Strep. rochei* and their activities.

Peak No.	R_t (min.)	Name	Formula	Area (%)	Activity	Reference
1	5.36	Octanoic acid	C8H14O2	1.37	Antifungal	Elhosari *et al.*, 2022
2	5.47	Vinyl-tetrahydro-4-pyranol4	C7H12O2	1.23	Antifungal	Hashem *et al.*, 2022
3	5.62	2-Hexadecanol (CAS)	C7H13N3O3S	1.25	Nematicidal	Madhavan, 2021
4	10.99	3-Methyl pentanoic acid	C6H12O2	1.09	Antifungal	Poveda, 2021
5	17.50	Iridomyrmecin	C10H16O2	17.4	Antifungal	Karsli and Shain 2021
6	17.73	2-Decenal, (E)-	C10H18O	2.77	Antifungal	Vidya *et al.*, 2022
7	17.12	Jasmonic acid	C12H18O3	3.89	Antifungal	Lee *et al.*, 2022
8	20.12	1-Tridecanol (CAS)	C13H28O	2.41	Antifungal	Netzker *et al.*, 2022
9	20.52	2-Dodecenal	C12H22O	8.26	Antibacterial, antifungal, Nematicidal	Vidya *et al.*, 2022
10	35.17	Hexadecanoic acid, trimethylsilyl ester (CAS)	C19H40O2Si	55.92	Antimicrobial, antigenotoxicity and antifungal	Saddiq *et al.*, 2022
11	42.14	Dodecachloro-3,4-benzo phenanthrene	C18C112	1.14	Antifungal	Del Tito *et al.*, 2020
12	53.48	3,4,5,6-Tetrakis (p-chloro phenoxy)-1,2-dicyanoben zene	C26H44O5	0.78	Antifungal	Alqahtani *et al.*, 2022

These data are compatible with those of Ayed *et al.* (2021), who discovered 14 chemicals, including three significant terpenoids, in *Streptomyces lydicus* following a VOC analysis: Three phenolic compounds, 3-carene-2,5-dione, geosmin, beta-cubebene, and one phenol, 2-(1,1-dimethylethyl)-6-methyl, made up 43 %, 20 %, 11 %, and 9.34 %, respectively, of the total VOCs generated. VOCs significantly reduced the mycelial development of *Sclerotium rolfsii, Fusarium solani*, *Rhizoctonia solani*, and *Fusarium oxysporum*. Additionally, most of these VOC compounds have a history of biological activity reports, including DPPH antioxidant, antibacterial, antiviral, antifungal, anticancer, and cytotoxic properties against various bacteria that produce biofilms (Kemung *et al.*, 2020). Finally, we can confirm that *Strep. rochei* is a unique producer of secondary metabolites, which have been proven effective in inhibiting plant pathogenic fungi, the most important of which are volatile organic compounds as well as the fungal cell wall-degrading enzymes. Hence, the success of our idea of using endophytic actinomycetes as biocontrol agents is a starting point for promising research toward the production of unconventional and environmentally safe biocides.

Conclusion

The current study showed that the endophytic actinomycete, *Streptomyces rochei* OM182844, is a suitable inhibitor for soil-borne pathogenic fungi directly or indirectly via bioactive metabolites, especially antifungal compounds. Cell wall-degrading enzymes such as chitinase and cellulase, as well as volatile organic compounds (VOCs) are considered the most essential metabolites discovered. GC-MS analysis recorded twelve VOCs in the cell-free filtrate of *Strep. rochei* and identified as an antifungal, the most abundant of which was Hexadecanoic acid, a trimethylsilyl ester (CAS), which yielded at a rate of more than fifty percent and was found to be an antifungal, antimicrobial and anti-genotoxicity compound. In sum, our results confirmed previous findings that *Strep. rochei* is an auspicious producer of these VOCs, an unusual class of secondary metabolites that have shown a potent ability to eliminate pathogenic fungi. Therefore, the discovery of new biocontrol agents as an alternative method to synthetic pesticides should be the future goal of keeping the environment clean and safe.

REFERENCES

Abdel-Aziz, M.S.; Ghareeb, M.A.; Hamed, A.A.; Rashad, E.M.; El-Sawy, E.R.; Saad, I.M. and Ghoneem, K.M. (2021). Ethyl acetate extract of *Streptomyces* spp. isolated from Egyptian soil for management of *Fusarium oxysporum*: the causing agent of wilt disease of tomato. *Biocatalysis and Agricultural Biotechnology*, 37, 102185.

Al Raish, S.M.; Saeed, E.E.; Alyafei, D.M.; El-Tarabily, K.A. and AbuQamar, S.F. (2020). Evaluation of *streptomyces* actinobacterial isolates as biocontrol agents against royal poinciana stem canker disease caused by *Neoscytalidium dimidiatum*. *Biological control,* 164, 104783.

Alblooshi, A.A.; Purayil, G.P.; Saeed, E.E.; Ramadan, G.A.; Tariq, S.; Altaee, A.S. and AbuQamar, S.F. (2022). Biocontrol potential of endophytic actinobacteria against *Fusarium solani,* the Causal Agent of Sudden Decline Syndrome on Date Palm in the UAE. *Journal of Fungi,* 8(1),8.

Alqahtani, S.S.; Moni, S.S.; Sultan, M.H.; Bakkari, M.A.; Madkhali, O.A.; Alshahrani, S.; and Sayed-Ahmed, M.Z. (2022). Potential bioactive secondary metabolites of *Actinomycetes* sp. isolated from rocky soils of the heritage village Rijal Alma, Saudi Arabia. *Arabian Journal of Chemistry*, 15(5), 103793.

Ayed, A.; Kalai-Grami, L.; Ben Slimene, I.; Chaouachi, M.; Mankai, H.; Karkouch, I. and Limam, F. (2021). Antifungal activity of volatile organic compounds from *Streptomyces* sp. strain S97 against *Botrytis cinerea*. *Biocontrol Science and Technology,* 31(12), 1330-1348.

Corral, D.A.P.; Paz, J.D.J.O.; Orozco, G.I.O.; Muñiz, C. H. A.; Marina, M.Á.S.; Cisneros, M.F.R. and Velasco, C.R. (2022). Antagonistic effect of volatile and non-volatile compounds from *Streptomyces* strains on cultures of several phytopathogenic fungi. *Emirates Journal of Food and Agriculture*, pp, 879-889.

de Brito, M.V.; Fonseca, W.L.; Mafezoli, J.; Barbosa, F. G.;Nunes, F.M.; de Mattos, M.C. and Oliveira, M.C. (2022). Biologically active volatile organic compounds (VOCs) produced by rhizospheric actinobacteria strains inhibit the growth of the phytopathogen *Colletotrichum musae*. *Journal of the Brazilian Chemical Society,* pp, 1-18.

Del Tito, A.; Abdulla, H.O.; Ravelli, D.; Protti, S. and Fagnoni, M. (2020). Photocatalyzed syntheses of phenanthrenes and their aza-analogues. A review. *Beilstein Journal of Organic Chemistry, 16*(1), 1476-1488.

El-Akshar, E.A., El-Meihy, R.M., Tewfik, T.A., Youssef, A.M. and Abou-Aly, H.E. (2022). Chitinolytic activity and antifungal potential of endophytic actinobacteria isolated from the cucumber plant. (In press).

El-Boghdady, M.M.E. (1993). Integrated post-harvest diseases management of certain pome fruits. Ph.D. Thesis, Fac. Agr., Al-Azhar Univ., Egypt.

Elhosari, D.; Abdou, M.; Abdel-Razek, A. S.; Hamed, A.A.; Shaaban, M., and El-Gindi, M. (2022). Preliminary investigation of the fungal endophytic extract isolated from *Tabernaemontana pandacaqui* leaves and evaluation of its antioxidant and cytotoxic potentials. *Journal of Advanced Pharmacy Research,* 6(2), 68-77.

Garbeva, P.; Hordijk, C.; Gerards, S. and de Boer, W. (2014). Volatile-mediated interactions between phylogenetically different soil bacteria. *Frontiers in Microbiology* , 5, Article ID 289.

Gebily, D.A.; Ghanem, G. A.; Ragab, M.M.; Ali, A.M.; Soliman, N.E.D.K.; El-Moity, A. and Tawfik, H. (2021). Characterization and potential antifungal activities of three *Streptomyces* spp. as biocontrol agents against *Sclerotinia sclerotiorum* (Lib.) de Bary infecting green bean. *Egyptian Journal of Biological Pest control,* 31(1), 1-15.

Gu, Y.Q.; Mo, M.H.; Zhou, J.P.; Zou, C.S.; Zhang, K.Q. (2007) Evaluation and

identification of potential organic nematicidal volatiles from soil bacteria. *Soil Biology and Biochemistry*, 39, 2567-2575.

Hashem, A.H.; Shehabeldine, A.M.; Abdelaziz, A.M.; Amin, B.H. and Sharaf, M.H. (2022). Antifungal activity of endophytic *Aspergillus terreus* extract against some fungi causing mucormycosis: ultrastructural study. *Applied Biochemistry and Biotechnology*, pp, 1-15.

Janatiningrum, I., and Lestarl, Y. (2022). Enzyme production, antibacterial and antifungal activities of actinobacteria isolated from *Ficus deltoidea* rhizosphere. *Biodiversitas Journal of Biological Diversity*, *23*(4), 1950-1957.

Kanini, G.S., Katsifas, E.A., Savvides, A.L. and Karagoun, A.D. (2013) *Streptomyces rochei* ACTA1551, an indigenous Greek isolate studied as a potential biocontrol agent against *Fusarium oxysporum* f.sp. *lycopersici*. *BioMed Research International*, pp, 1-10, Article ID 387230.

Karsli, A. and Şahin, Y.S. (2021). The role of fungal volatile organic compounds (FVOCs) in biological control. *Türkiye Biyolojik Mücadele Dergisi*, 12(1), 79-92.

Kemung, H.M.;Tan, L.T.H.; Chan, K.G.; Ser, H.L.; Law, J.W.F.; Lee, L.H. and Goh, B.H, (2020) *Streptomyces* sp. strain MUSC 125 from mangrove soil in Malaysia with anti-MRSA, anti-biofilm and antioxidant activities. *Molecules,* 25(3545), 1-20.

Lee, J.H.; Anderson, A.J. and Kim, Y.C. (2022). Root-associated bacteria are biocontrol agents for multiple plant pests. *Microorganisms*, 10(5), 1053.

Lee, S.K., Lee, J.H., Kim, H.R. Chun, Y., Lee, J.H., Park, C., Yoo, H.Y., Kim, S. W. (2021) Rapid and concise quantification of mycelial growth by microscopic image intensity model and application to mass cultivation of fungi. *Scientific Reports*, 11, Article ID 24157.

Liu, D.; Yan, R.; Fu, Y.; Wang, X.; Zhang, J. and Xiang, W. (2019). Antifungal, plant growth promoting and genomic properties of an endophytic actinobacterium *Streptomyces* sp. NEAU-S7GS2. *Frontiers in Microbiology*, 10, Article ID 2077.

Madhavan, S.A. (2021). In-vitro pharmacological and GC-MS analysis of bioactive compounds presents in *Bytteneria herbaceae*. *International Journal of Zoological and Entomological Letters*, 2021; 1(1), 20-26

Miller, G.L. (1959). Use of dinitro salicylic acid reagent for determination of reducing sugars. *Analytical Chemistry*, 31, 426-428.

Mitra, D., Mondal, R.; Khoshru, B.; Senapati, A.; Radha, T.K., Mahakur, B. and Mohapatra, P. K. D. (2022). Actinobacteria-enhanced plant growth, nutrient acquisition, and crop protection: Advances in soil, plant, and microbial multifactorial interactions. *Pedosphere*, 32(1), 149 -170.

Neethu, K.; Rubeena, M.; Sajith, S.; Sreedevi, S.; Priji, P.; Unni, K.N., and Benjamin, S. (2012). A novel strain of *Trichoderma viride* shows complete lignocellulolytic activities. *Advances in Bioscience and Biotechnology*, 3, 1160-1166.

Netzker, T.; Shepherdson, E.M.; Zambri, M.P. and Elliot, M.A. (2020). Bacterial volatile compounds: functions in communication, cooperation, and competition. *Annual Review of Microbiology*, 74, 409-430.

Poveda, J. (2021). Beneficial effects of microbial volatile organic compounds (MVOCs) in plants. *Applied Soil Ecology*, 168, Article ID 104118.

Rahman, M.A.; Begum, M.F. and Alam, M.F. (2009). Screening of *Trichoderma* isolates as a biological control agent against *Ceratocystis paradoxa* causing pineapple disease of sugarcane. *Mycobiology,* 37(4), 277-285.

Ruangwong, O.U.; Kunasakdakul, K.; Daengsuwan, W.; Wonglom, P.; Pitija, K., and Sunpapao, A. (2022). A *Streptomyces* rhizobacterium with antifungal properties against spadix rot in flamingo flowers. *Physiological and Molecular Plant Pathology,* 117, Article ID 101784.

Saddiq, A.A.; Tag, H.M.; Doleib, N.M.; Salman, A.S. and Hagagy, N. (2022). Antimicrobial, antigenotoxicity, and characterization of *Calotropis procera* and its rhizosphere-inhabiting actinobacteria: In Vitro and In Vivo Studies. *Molecules*, 27(10), 3123.

Sadeghi, A.; Karimi, E.; Dahaji, P.A.; Javid, M.G.; Dalvand, Y. and Askari, H. (2012). Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. *World Journal of Microbiology and Biotechnology*, 28(4), 1503-1509.

Schoffelmeer, E.A.M.; Klis, F.M.; Sietsma, J.H. and Cornelissen B.J.C. (1999). The cell wall of *Fusarium oxysporum*. *Fungal Genetics and Biology*, 27, 275-282.

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

Singh, A.K.; Tripathi, V.; Shukla, A.K. and Kumar, P. (2022). Bacterial endophytes for sustainable agriculture and Environmental Management. Springer Nature Singapore Pte Ltd., pp, 95-116.

Tilocca, B.; Cao, A. and Migheli, Q. (2020). Scent of a killer: Microbial volatilome and its role in the biological control of plant pathogens. *Frontiers in Microbiology,* 11, 1-13.

Vidya, V.R.;Pillai, P. and Preetha, T.S. (2022). Comparative chemical profiling of essential oil components using GC-MS in micro, mini and mother rhizomes of *Kaempferia galanga* L. *Journal of Advanced Scientific Research*, 13(01), 188-199.

Yang, M.L.; Lu, J.; Pang, Y.; Hu, Q.; Guo, Z.L and Wang, C. (2019). Biocontrol activity of volatile organic compounds from μ αβα TD-1 against γ α growth and aflatoxin production. *Journal of Microbiology*, 57, 396-404.