AJAB

Asian J Agric & Biol. xxxx(x).
DOI: 10.35495/ajab.2024.088

Original Article

Antifungal efficacy of *Streptomyces murinus* against postharvest pathogens *Penicillium digitatum* and *Penicillium italicum* in oranges

Thi Thuy Tien Nguyen[1]*, Hien Trang Nguyen[1], Thanh Long Le [1], Thy Dan Huyen Nguyen[1]
[1]Department of Engineering and Food Technology, University of Agriculture and Forestry, Hue University, Hue, Thua Thien Hue, 530000, Vietnam.

Received:
May 18, 2024
Accepted:
September 12, 2024
Published Online:
November 11, 2024

**Corresponding author email:*
ntttien@hueuni.edu.vn

Abstract

Forty-eight *Streptomyces* strains were screened for antifungal activities against *P. digitatum* P2 and *P. italicum* I6 isolated from oranges infected with green mold and blue mold diseases, respectively, using the dual culture method. Among 48 screened strains of *Streptomyces*, *S. murinus* NARZ showed the strongest activity by completely inhibiting the growth of both pathogens. The antifungal activities of *S. murinus* NARZ were evaluated based on the percentage inhibition of radial growth (PIRG, %) using culture filtrate (CF) and ethyl acetate crude extract. The EC_{50} values of the 7-day *S. murinus* CF were 13.25% and 33.72% against *P. digitatum* P2 and *P. italicum* I6, respectively. The EC_{50} and MIC_{90} values of the crude extract were 45.03 µg/mL and 193.15 µg/mL for *P. digitatum* P2, and 68.25 µg/mL and 295.60 µg/mL for *P. italicum* I6, respectively. Notably, out of the six International Streptomyces Project Media (ISP2, ISP3, ISP4, ISP4G, ISP4Y, and ISP8), ISP3 medium was optimal for *S. murinus* NARZ cultivation to produce antifungal metabolites, with 50% CF yielding 100% inhibition against both *Penicillium* pathogens. Furthermore, the CF had notable thermal resistance; the PIRGs decreased from 100% to 74.22% for *P. digitatum* P2 and to 64.59% for *P. italicum* P6 after treatment at 121°C for 15 min. The tests showed that oranges sprayed with 100% CF of *S. murinus* NARZ remained symptom-free of blue and green mold diseases after a seven-day incubation following artificial inoculation with *Penicillium* species. This study highlights the potential of *Streptomyces* strains, particularly *S. murinus* NARZ, as effective biocontrol agents against postharvest fungal diseases in oranges.

Keywords: Antifungal activities, Biocontrol, Postharvest diseases, *Penicillium*, *Streptomyces murinus*

How to cite this:

Nguyen TTT, Nguyen HT, Le TL and Nguyen TDH. Antifungal efficacy of *Streptomyces murinus* against postharvest pathogens *Penicillium digitatum* and *Penicillium italicum* in oranges. Asian J. Agric. Biol. xxxx(x): 2024088.. DOI: https://doi.org/10.35495/ajab.2024.088

Introduction

Citrus fruits are globally valued as an important source of their rich health-related nutrients, such as carotenoids, flavonoids, pectin, calcium, potassium, phytonutrients, phenolic compounds, vitamin C, soluble and insoluble fibers (Abobatta, 2019). Regardless of their significance, citrus fruits are vulnerable to postharvest diseases that result in huge economic losses. Notably, blue mold and green mold diseases, caused by *Penicillium italicum* and *P. digitatum*, respectively, contribute to estimated postharvest losses ranging from 10% to 90% (Shojaee et al., 2014; Wang et al., 2022).

After invading the fruit, these fungal pathogens produce natural acids, reducing the pH level to align with the ideal range for their enzymes that break down cell walls, such as polygalacturonases. Additionally, citrus volatiles have been suggested to assist the development of *P. digitatum* (Ladaniya, 2008). While *P. digitatum* infects citrus via mechanical wounds, affecting individual fruits (Bhatta, 2022), *P. italicum* targets healthy citrus directly and releases enzymes that soften neighboring fruits, aiding in fungal entry (Ladaniya, 2008). Initial infection of citrus results in small water-soaked lesions with white mycelia bordered by a ring of rotting pericarp. These lesions then expand, and spores adopt a green for *P. digitatum* or a bluish hue for *P. italicum* (Ladaniya, 2008; Wang et al., 2022). Ultimately, the infection spreads throughout the mericarp of the fruit, leading to its complete decay (Bhatta, 2022).

The primary risks posed by *Penicillium* species stem from their spores, which appear as fine powders that are readily air-borne (Ladaniya, 2008), along with their swift disease cycle, typically 3–5 days at 25°C (Bhatta, 2022). Synthetic fungicides have been used extensively to tackle blue and green mold postharvest citrus diseases. As public concerns regarding human health, food safety, and environmental effects increase, there's a growing interest in discovering eco-conscious management approaches. An attractive strategy is biological control, which addresses postharvest diseases using antagonistic microorganisms, offering a promising alternative approach (Bhatta, 2022; Wang et al., 2022). Antagonistic microbes such as *Pseudomonas fluorescens* (Wang et al., 2018), *Clavispora lusitaniae* (Perez et al., 2019), *Aureobasidium pullulans* (Freimoser et al., 2019) , *Streptomyces* spp. (Shojaee et al., 2014), *S. philanthi* (Boukaew et al., 2020) have been employed to manage *P. italicum* and *P. digitatum*. Notably, the *Streptomyces* genus stands out as a potent crop protection agent because of its bioactive secondary metabolites that possess antibacterial, antifungal, anti-inflammatory, and antitumor properties (Ayed et al., 2021; Hasani et al., 2014; Shojaee et al., 2014). Procópio et al. (2012) reported that up to 80% of antibiotics have been derived from the *Streptomyces* genus, with over 10,000 bioactive compounds (Ayed et al., 2021).

The current study aimed to screen the antifungal properties of 48 *Streptomyces* specimens isolated from soil samples in Vietnam against *P. digitatum* P2 and *P. italicum* I6. The selected strain underwent diverse *in vitro* tests to evaluate its antifungal characteristics regarding the culture filtrate and crude extract. Furthermore, experiments have investigated its potential as an antagonist of blue and green mold diseases in orange fruits.

Material and Methods

Material

Forty-eight *Streptomyces* spp. isolates obtained from various soil samples in five provinces in Vietnam, including Thua Thien Hue, Gia Lai, Nghe An, Thanh Hoa, and Quang Nam, were screened for antifungal activities against the phytopathogenic fungi *P. italicum* and *P. digitatum*. Oranges, free of injuries or diseases, uniformly shaped, and sized, were handpicked from Nam Dong district, Thua Thien Hue province, Vietnam. The specimens were quickly carried to the laboratory and used within 24 hours.

Fungal isolation

Oranges showing typical symptoms of blue and green mold diseases were procured from Dong Ba market, Thua Thien Hue province, Vietnam, and used to isolate *P. italicum* and *P. digitatum* following previous descriptions with slight modifications (El-majberi et al., 2020; Ahmad et al., 2023). From the greenish and bluish lesion surface on oranges, spores of *Penicillium* pathogens were picked and placed into Petri plates using sterilized toothpicks. The Petri plates contained PDA (potato dextrose agar) supplemented with chloramphenicol (250 mg per liter) to prevent bacterial growth. The plates were incubated at 25-28^{o}C until typical colonies appeared. Suspected *Penicillium* isolates were subcultured and purified using the single isolation technique (Noman

et al., 2018). Those isolates were preliminary screened as *P. italicum* and *P. digitatum* based on their morphologies according to descriptions of Pitt and Hocking, 2009 and Palou, 2014. Finally, the isolates were identified by analyzing their ITS (Internal Transcribed Spacer) region using the Sanger sequencing method with the primer set (ITS1, 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4, 5′-TCCTCCGCTTATTGATATGC-3′) (Zarrin et al., 2016), using the service of a DNA SEQUENCING company (Can Tho, Vietnam). Resulting sequences underwent BLASTn searches against databases deposited in GenBank (Altschul et al., 1990) to identify their taxonomy. A phylogenetic tree was built based on sequences of isolates in this study and reference sequences obtained from GenBank databases using MEGA11 software (Tamura et al., 2021). All sequences were aligned and equalized with ClusterW. The neighbor-joining phylogenetic tree was generated with pairwise gap deletion. *Aspergillus oryzae* NRRL 447 (NR 135395.1) was used as an outgroup.

Screening for the antifungal ability of *Streptomyces* sp.

The antifungal abilities of 48 *Streptomyces* spp. were screened using a dual culture method with minor modifications (Dezfully and Ramanayaka, 2015). *P. italicum* and *P. digitatum* were independently cultured on PDA medium. Each *Streptomyces* sp. was streaked (1 cm) in a straight line on ISP4 agar across the equator of the Petri dish and then incubated for 96 h at 28 ºC. The ISP4 medium comprised soluble starch (10 g/L), $CaCO_3$ (2 g/L), NaCl (1 g/L), $(NH_4)_2SO_4$ (2 g/L), $MgSO_4.7H_2O$ (1 g/L), K_2HPO_4 (1 g/L), $ZnSO_4.7H_2O$ (0.001 g/L), $MnCl_2.7H_2O$ (0.001 g/L), and agar (20 g/L). Two pieces (5 mm) from growing pure colonies of each fungus were independently placed on either side of the *Streptomyces* streak at the midpoint between the streak and edge of the plate.

The antifungal efficacy of *Streptomyces* strains was determined by assessing the growth of *P. italicum* and *P. digitatum* after seven-day incubation at 28°C (Azish et al., 2020). Inhibition levels were categorized as follows: very strong (+++), demonstrating complete inhibition of *P. italicum* and *P. digitatum* growth; strong (++), indicating fungi could survive on the agar pieces but could not reach the ISP4 agar; weak (+), suggesting slight growth but failed to reach the *Streptomyces* streak; and none (-), indicating healthy growth of fungi. The strain exhibiting the most robust inhibition was selected for further testing.

Antifungal activity of culture filtrate obtained from the selected *Streptomyces* strain

The selected *Streptomyces* strain was cultured in ISP4 medium at 28-30ºC for seven days with shaking at 100 rpm. The culture filtrate (CF) was then obtained by centrifuging the mixture for five minutes at 5,000 rpm and sterilizing the supernatant with a 0.22 µm membrane (Jacob et al., 2017). The CF was aseptically added to Petri dishes containing PDA medium, generating six dilutions, representing specific concentrations from 50% CF to 0% (control) at 10% intervals. A 5 mm piece of each fungus was excised from the edge of the pure colony and individually placed in the center of the prepared Petri dishes. This process was repeated thrice for each concentration of the CF. The radial growth diameters of fungal colonies on Petri plates were measured, and each fungus's average growth rate (mm/day) was documented. After seven days, the formula PIGR (%) = (C – T) × 100/C was used to compute the percentage inhibition of radial growth (PIRG, %), where C and T stand for the colony diameters of the control and tested concentration (mm), respectively. Additionally, the half-maximal effective concentration (EC_{50}) was determined (Jacob et al., 2017; Qi et al., 2019).

Effect of cultivation media on antifungal activities of the selected *Streptomyces* strain

The selected *Streptomyces* strain was cultured on ISP4 agar at 28-30ºC for five days, and the cells were collected using 1 mL of sterile distilled water in Eppendorf tubes. After vortexing, 100 µL of the prepared *Streptomyces* cell suspension was added to 250-mL flasks, each containing 100 mL of six distinct media: ISP2, ISP3, ISP4, ISP4G, ISP4Y, and ISP8, with three replicates per medium. The media were prepared following previously outlined procedures (Shirling and Gottlieb, 1966). The ISP2 medium contained malt extract (10 g/L), yeast extract (4 g/L), and dextrose (4 g/L). ISP3 medium included $FeSO_4$.$7H_2O$ (0.1 g/L), oatmeal (20 g/L), $MnCl_2$.$4H_2O$ (0.1 g/L), and $ZnSO_4$.$7H_2O$ (0.1 g/L). The ISP4G and ISP4Y media contained the same components as the ISP4 medium (mentioned above), with the addition of glucose (20 g/L) and yeast extract (1 g/L), respectively. The ISP8 medium contained peptone (5 g/L), KNO_3 (1 g/L), and meat

extract (3 g/L). The protein sources for these media were purchased from HiMedia (India).
Subsequently, the culture flasks were shaken at 100 rpm at 28-30ºC for seven days, and their CFs were independently added to Petri dishes containing PDA medium, achieving a concentration of 50% CF. Control plates contained PDA medium without any CF addition. The effect of cultivation media on antifungal activity was evaluated based on PIRG (%) against *P. italicum* and *P. digitatum* (Jacob et al., 2017; Qi et al., 2019). Cells collected from each medium were dried at 55°C until their weight was stable (Bundale et al., 2015). The medium with the highest antifungal activity, as indicated by CF, was chosen for subsequent experiments.

Effect of thermal treatment on antifungal activity
CFs obtained from the selected cultivation medium underwent heat treatment at various temperatures: 30, 60, 90, and 121°C for 15 min, replicated thrice per temperature. These treated CFs were evaluated for the effect of thermal treatment on their antifungal efficacy at a concentration of 50% by calculating the PIRG (%) against *P. italicum* and *P. digitatum* (Chen et al., 2021).

Antifungal activities of crude extract obtained from the selected *Streptomyces* strain
The CF obtained from the selected cultivation medium was mixed with 99% ethyl acetate (Duksan, Korea) in an equal volume, shaken for 45 min, and left to settle for 15 min, leading to separation into two distinct layers. The upper organic layer was collected, while the lower aqueous suspension was subjected to another round of mixing with ethyl acetate and iterated to ensure complete extraction of all antifungal compounds. The upper layers were combined and concentrated using a vacuum rotary evaporator (Buchi Rotavapor R-100) at 40°C and 338 mbar vacuum. The resulting extract was dried using a lyophilizer (Alpha 1-2 LSCbasic, Christ) to yield crude extract (CE) (Jacob et al., 2017).
The antifungal activity of CE was assessed relying on the average growth rate (mm/day) and PIRG (%) against both fungi on PDA medium plates, supplemented with varying CE concentrations: 0 (control), 12.5, 50, 100, 200, and 400 µg/mL. PDA plates containing nystatin (10 µg/mL, MedChemExpress, USA) were positive controls. Eachexperiment was replicated thrice for each tested CE concentration. Finally, EC_{50} and minimum inhibitory concentrations (MIC_{90}) were determined.

Antifungal activities of the CF against green mold and blue mold diseases on orange fruits
Antagonism assays were carried out according to previously described methods with minor modifications (Boukaew et al., 2020; Shojaee et al., 2014). One mL of sterile distilled water was added to Petri plates containing seven-day-old *P. italicum* and *P. digitatum* independently. The spore suspension was collected through a four-layer sterile cheesecloth and was adjusted to 10^5 spores/mL for use as the inoculum (Boukaew et al., 2020).
The oranges underwent a double wash with tap water, followed by immersion in NaOCl (2% v/v) for 3–5 minutes, and then washed with sterile distilled water. The oranges were wiped using sterile tissues to remove excess water from their surfaces. On the equator of each orange, two opposing wounds (2 mm depth and 2 mm width) were created using a sterile needle (Boukaew et al., 2020).
The CF was diluted with sterilized distilled water to obtain various concentrations, including 0%, 10%, 20%, 40%, 50%, and 100% CF. Each orange was sprayed with 30 mL of the prepared CF preparation at a distance of 20 cm from the fruits to make a thin mist and dried at room temperature for one hour (Shojaee et al., 2014). The positive control was Fosetyl aluminum (5 µg/mL, Bayer), which underwent the same treatment as the CF applied to oranges.
These oranges were artificially inoculated with 10 µL of each prepared *P. italicum* and *P. digitatum* inoculum into the wounds. After allowing 30 min for drying, each fruit was placed in a plastic box with dimensions of 200 × 130 × 50 mm and then kept at room temperature. These boxes contained sterile water-moistened tissues to maintain humidity. This procedure was replicated thrice for each tested CF concentration and for each tested fungus. Each fruit's lesion diameter (mm) was recorded after three days using a digital micrometer every two days for seven days. The antifungal properties of CF were determined using the formula PIRG (%) = $(R_1 – R_2)/R_1$, where R_1 and R_2 represent the lesion diameters of control orange fruits and oranges treated with CF concentrations, respectively (Yong et al., 2022).

Statistical analysis
The experimental data were statistically analyzed using the SPSS program (v. 20.0) via one-way ANOVA (Duncan's test) with a significance level set at $p<0.05$.

Results

Fungal isolation

During fungal isolation, isolates showing typical *P. italicum* and *P. digitatum* morphologies were subcultured and purified. Colonies showing olive green surface, flat, powdery texture, and free of exudate droplets with cream-yellow on the reverse side were suspected as *P. digitatum*. On the other hand, colonies showing bluish-gray, planar, velutinous, heavy sporing without exudate droplets and enclosed by a white mold ring with brown on the reverse were suspected as *P. italicum*. Under microscopic observation, conidia of suspected *P. digitatum* and *P. italicum* were ellipsoidal to cylindrical or subglobose, smooth-walled with irregular branches attaching spores on the tips, measuring 2.3-2.7 × 3.7-4.2 µm in size (Figure-1). The morphological characteristics observed were consistent with the descriptions provided by Palou (2014) and Pitt and Hocking (2009) for *P. italicum* and *P. digitatum*. After preliminary screening based on fungal morphologies, representative isolates of suspected *P. digitatum* (isolate P2) and *P. italicum* (isolate I6) were sequenced in their ITS region. The BLASTn searches indicated that isolate P2 was 100% identical to *P. digitatum* PD-038 (MT573500.1) and the isolate I6 100% matched *P. italicum* aT3 (KU561924.1). Furthermore, the phylogenetic analysis indicates that the isolate *P. italicum* I6 and *P. digitatum* P2 were closest to *P. italicum* aT3 and *P. digitatum* PD-038, respectively (Figure-2). Therefore, they were identified as *P. digitatum* P2 and *P. italicum* I6 and used for further experiments. The ITS sequences of *P. digitatum* P2 and *P. italicum* I6 were submitted to GenBank and assigned the accession numbers PP737844 and PP737843, respectively.

Screening for antifungal activities of Streptomyces sp.

The 48 tested *Streptomyces* strains exhibited varying levels of antifungal efficacy (**Error! Reference ource not found.**). Among these strains, only one strain (2.10%) inhibited the growth of *P. digitatum* P2 and *P. italicum* I6. Eight strains (16.67%), 20 strains (41.66%), and three strains (6.25%) showed strong, weak, and no antifungal activity, respectively, against both *Penicillium* fungi. Three strains (6.25%), namely NB2.01, NBA.01, and NYD.01, displayed strong antifungal activity against *P. digitatum* P2 but showed weak activity against *P. italicum* I6. In contrast, four strains (8.33%), including G25.105, NT2.01, NBA.03, and NV4.06, showed strong antifungal activity against *P. italicum* I6 but had weaker effects on *P. digitatum* P2. Five strains (10.42%), listed as H12, H16, ND2.12, HT125, and NNY.01, demonstrated weak antifungal activity against *P. digitatum* P2, but none against *P. italicum* I6. Meanwhile, four strains, NBY.08, NTY.04, NBA.02, and NBY.03, accounting for 8.33%, did not have antifungal activities against *P. digitatum* P2 but weak against *P. italicum* I6.

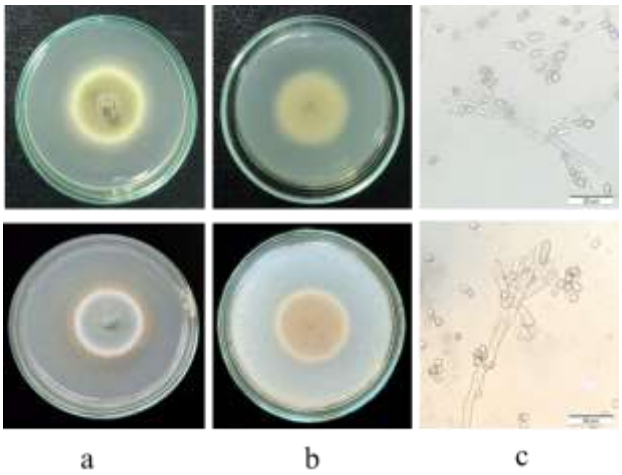

Figure-1. Morphological characteristics of P. digitatum P2 (top row) and P. italicum I6 (bottom row). a: colony observed from the top side of Petri dish, b: colony observed from the bottom side of Petri dish, c: micrographs of conidia

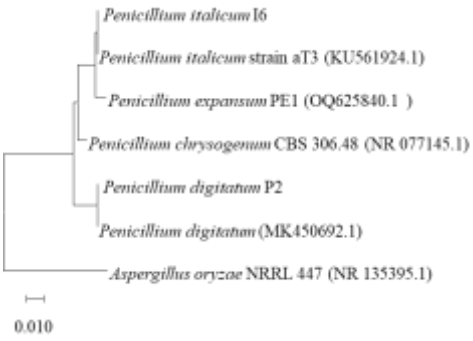

Figure-2. A phylogenetic tree indicates a relationship between isolates *P. italicum* I6 and *P. digitatum* P2 with other reference strains based on their sequences of ITS region using the Neighbor-Joining method by MEGA11. Accession numbers of reference strains are in parentheses. Scale bar: 0.010 substitutions per nucleotide position.

Table-1. Antifungal activities of *Streptomyces* spp. against *P. digitatum P2* and *P. italicum I6*

No	*Streptomyces* ID	Antifungal activities		No	*Streptomyces* ID	Antifungal activities	
		P. digitatum P2	*P. italicum* I6			*P. digitatum* P2	*P. italicum* I6
1	G25.105	+	++	25	NBY.08	-	+
2	H12	+	-	26	ND2.03	++	++
3	H16	+	-	27	ND2.12	+	-
4	HC111	+	+	28	ND4.01	++	++
5	HC6	+	+	29	NN2.03	-	-
6	HDM.32	+	+	30	NN4.01	-	-
7	HDM1.22	+	+	31	NN4.14	++	++
8	HN1.11	+	+	32	NNA.03	+	+
9	HT123	-	-	33	NBY.05	+	+
10	HT125	+	-	34	NNY.01	+	-
11	HTD.03	+	+	35	NNY.02	+	+
12	ISP5069	+	+	36	NNY.08	+	+
13	N2B1	++	++	37	NT2.01	+	++
14	N2R3	++	++	38	NT4.03	+	+
15	N2R4	++	++	39	NT4.13	+	+
16	N4B5	+	+	40	NTA.01	+	+
17	N4R1	++	++	41	NTA.03	+	+
18	NARZ	+++	+++	42	NTY.03	++	++
19	NB2.01	++	+	43	NTY.04	-	+
20	NBA.01	++	+	44	NV2.03	+	+
21	NBA.02	-	+	45	NV2.04	+	+
22	NBA.03	+	++	46	NV4.04	+	+
23	NYD.01	++	+	47	NV4.06	+	++
24	NBY.03	-	+	48	NVA.01	+	+

Note: (+++), (++), (+), and (-) indicate very strong, strong, weak, and none antifungal activity, respectively.

Of the 48 strains, NARZ exhibited the strongest activity in preventing the growth of both fungi and was selected for comprehensive evaluation of its antifungal attributes. This strain was previously identified as *Streptomyces murinus* in our previous publication (Nguyen et al., 2022).

Effect of *S. murinus* NARZ on controlling *P. digitatum* P2 and *P. italicum* I6

The growth rate of *P. digitatum* P2 was consistently lower than that of *P. italicum* I6 at all tested CF doses (**Error! Reference source not found.**). Without CF supplementation, the growth rates were 5.72 mm/day and 6.11 mm/day in diameter for *P. digitatum* P2 and *P. italicum* I6, respectively. With the addition of 50% CF from *S. murinus* NARZ, a significant reduction in growth rate was observed, dropping to 1.51 mm/day and 2.02 mm/day in diameter for *P. digitatum* P2 and *P. italicum* I6, respectively.

Table-2. Effect of the CF concentrations on growth rate and PIRG of *P. digitatum* P2 and *P. italicum* I6

CF concentration %	Growth rate (mm/day)		PIRG (%)	
	P. digitatum P2	*P. italicum* I6	*P. digitatum* P2	*P. italicum* I6
0	5.72 ± 0.33^{a}	6.11 ± 0.05^{a}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}
10	3.18 ± 0.13^{b}	4.98 ± 0.03^{b}	44.37 ± 1.71^{d}	18.53 ± 0.65^{e}
20	2.37 ± 0.14^{c}	4.20 ± 0.12^{c}	58.49 ± 0.35^{c}	31.2 ± 2.14^{d}
30	2.00 ± 0.21^{d}	3.41 ± 0.17^{d}	65.10 ± 2.06^{b}	44.28 ± 3.01^{c}
40	1.87 ± 0.23^{d}	2.93 ± 0.04^{e}	67.47 ± 2.17^{b}	52.18 ± 0.55^{b}
50	1.51 ± 0.05^{e}	2.02 ± 0.04^{f}	73.85 ± 0.68^{a}	66.90 ± 0.58^{a}

The data are shown as mean ± standard deviation. Varied letters within a column indicate a

statistically significant difference at $p < 0.05$, determined by Duncan's test.

Higher CF concentrations led to higher PIRG values. The EC_{50} values of CF for *P. digitatum* P2 and *P. italicum* I6 were determined to be 13.25% ($y = 17.527\ln(x) + 4.7185$; $R^2 = 0.9868$) and 33.72% ($y = 28.625\ln(x) - 50.701$; $R^2 = 0.9494$), respectively. At a 50% CF concentration, PIRG reached 73.85% and 66.90% for *P. digitatum* P2 and *P. italicum* I6, respectively. There was a statistically significant difference in the PIRG and growth rate ($p < 0.05$) for both *P. digitatum* P2 and *P. italicum* I6 at every interval of CF concentration level, except for *P. digitatum* P2 at 30% and 40%. Therefore, 50% CF was used for subsequent experiments for both tested pathogens.

Effect of cultivation media on antifungal activity of *S. murinus* NARZ

The CFs obtained from *S. murinus* NARZ cultivated in different media were added to Petri dishes containing PDA medium at a concentration of 50%. The results revealed that the composition of culture media significantly influenced the dried biomass of *S. murinus* NARZ and the antifungal activities of CF against *P. digitatum* P2 and *P. italicum* I6 (**Table-3** and Figure-3).

Table-3. Effect of cultivation media on antifungal activities of *S. murinus* NARZ and its dried biomass

Cultivation media	Growth rate (mm/day)		PIRG (%)		Dried biomass of *S. murinus* NARZ (mg/L))
	***P. digitatum* P2**	***P. italicum* I6**	***P. digitatum* P2**	***P. italicum* I6**	
Control (0% CF)	5.09 ± 0.30^{b}	5.93 ± 0.04^{a}	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}	
ISP2	5.58 ± 0.27^{a}	4.72 ± 0.04^{b}	0.00 ± 0.00^{f}	20.40 ± 0.65^{f}	1.81 ± 0.03^{e}
ISP3	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	5.17 ± 0.03^{b}
ISP4	1.20 ± 0.09^{f}	1.95 ± 0.15^{f}	76.25 ± 2.64^{b}	67.06 ± 2.55^{b}	6.38 ± 0.02^{a}
ISP4G	2.05 ± 0.17^{e}	2.17 ± 0.13^{e}	59.69 ± 2.20^{c}	63.30 ± 2.07^{c}	4.83 ± 0.01^{bc}
ISP4Y	3.91 ± 0.27^{c}	4.18 ± 0.09^{d}	23.23 ± 1.91^{e}	29.46 ± 1.68^{d}	4.67 ± 0.03^{c}
ISP8	2.75 ± 0.23^{d}	4.48 ± 0.19^{c}	45.96 ± 1.28^{d}	24.51 ± 2.86^{e}	3.27 ± 0.04^{d}

The data are shown as mean ± standard deviation. Varied letters within a column indicate a statistically significant difference at $p < 0.05$ (Duncan's test).

Cont ISP2 ISP3 ISP4 ISP4 IS ISP8

Figure-3. Morphologies of *P. digitatum* P2 (above) and *P. italicum* I6 (below) on PDA medium supplemented with 50% CF collected from various cultivation media of *S. murinus* NARZ. The control was PDA medium without the addition of CF.

The ISP2 medium was the least suitable for *S. murinus* NARZ growth and antifungal compound production. The dried biomass was only 1.81 mg/L, and the PIRGs reached 0.00% and 20,40% for *P. digitatum* P2 and *P. italicum* I6, respectively. Notably, *P. digitatum* P2 colonies grew more quickly in plates containing 50% CF of ISP2 medium than in control plates, with a growth rate of 5.58 mm/day and 5.09 mm/day, respectively. ISP4 was the most suitable for *S. murinus* NARZ biomass, measuring 6.38 mg/L. This medium also facilitated the accumulation of antifungal metabolites in *S. murinus* NARZ, with PIGRs of 76.25% and 67.06% for *P. digitatum* P2 and *P. italicum* I6, respectively. Incorporating glucose and yeast extract in ISP4, as seen in ISP4G and ISP4Y, reduced the dried biomass of *S. murinus* NARZ and its antifungal activities. The CF harvested from the ISP8 medium resulted in PIRGs of only 45.96% for *P. digitatum* P2 and 24.51% for *P. italicum* I6. Conversely, the ISP3 medium emerged as the most suitable medium for cultivating *S. murinus* NARZ for the robust production of potent antifungal compounds, and the PIRGs reached 100% for both *P. digitatum* P2 and *P. italicum* I6. Consequently, the ISP3 medium was chosen for further cultivation of *S. murinus* NARZ in subsequent experiments.

Effect of thermal treatment on antifungal activity of *S. murinus* NARZ

The CF of *S. murinus* NARZ cultivated in ISP3 medium for seven days underwent heat treatment at 30, 60, 90, and 121°C for 15 min before being

independently added to the PDA medium to obtain 50% CF. Interestingly, the antifungal properties of the CF remained stable after treatment at high temperatures. PIRGs were well-preserved, with PIRG of 98.29% and 83.07% for *P. digitatum* P2 and *P. italicum* I6, respectively, after being subjected to 90°C for 15 min. The PIRGs exhibited a slight reduction, reaching 74.22% for *P. digitatum* P2 and 64.59% for *P. italicum* I6, after treatment with CF at 121°C for 15 min (Table4).

Table-4. Effect of thermal treatment on PIRG (%) of the 50% CF from *S. murinus* NARZ against the two fungi

Temperature, °C	PIRG (%)	
	***P. digitatum* P2**	***P. italicum* I6**
30	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
60	100.00 ± 0.00^{a}	95.68 ± 0.72^{b}
90	98.29 ± 0.42^{b}	83.07 ± 2.33^{c}
121	74.22 ± 1.28^{c}	64.59 ± 1.49^{d}

The data are shown as mean ± standard deviation. Varied letters within a column signify a statistically significant difference at $p < 0.05$ (Duncan's test).

Antifungal activities of crude extract obtained from S. murinus NARZ

The crude extract of *S. murinus* NARZ significantly inhibited the growth of both *Penicillium* species. PIRGs were dose-dependent (Table 5). At 400 µg/mL of the extract, the growth of both fungi was completely inhibited. The EC_{50} and MIC_{90} values of *S. murinus* NARZ crude extracts against *P. digitatum* P2 and *P. italicum* I6 were calculated. The EC_{50} reached 45.03 µg/mL and 68.25 µg/mL and MIC_{90} were 193.15 µg/mL and 295.60 µg/mL for *P. digitatum* P2 and *P. italicum* I6, respectively. The equations were $y = 27.469\ln(x) - 54.579$ and $R^2 = 0.9636$ for *P. digitatum* P2 and $y = 27.788\ln(x) - 65.234$ and $R^2 = 0.9976$ for *P. italicum* I6. Nystatin (10 µg/mL) also inhibited the growth of *P. digitatum* P2 and *P. italicum* I6. Its efficiency against *P. digitatum* P2 was similar to 25 µg/mL of *S. murinus* NARZ extract. The colony morphologies of *P. digitatum* P2 and *P. italicum* I6 appeared smaller and denser when exposed to extracts from *S. murinus* NARZ.

Table-5. Effect of CE concentrations (µg/mL) on the growth rate of *P. italicum* and *P. digitatum*, and their PIRGs

CE concentration, µg/mL	Growth rate (mm/day)		PIRG (%)	
	***P. digitatum* P2**	***P. italicum* I6**	***P. digitatum* P2**	***P. italicum* I6**
0	5.06 ± 0.17^{a}	5.92 ± 0.16^{a}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}
12.5	4.43 ± 0.23^{b}	5.53 ± 0.22^{b}	12.46 ± 1.72^{f}	6.61 ± 1.15^{g}
25	3.61 ± 0.25^{c}	4.64 ± 0.23^{c}	28.66 ± 2.63^{e}	21.67 ± 1.89^{f}
50	2.18 ± 0.15^{d}	3.39 ± 0.21^{d}	56.95 ± 1.65^{d}	42.76 ± 1.98^{e}
100	1.08 ± 0.14^{e}	2.14 ± 0.13^{f}	78.69 ± 2.16^{c}	63.9 ± 1.22^{c}
200	0.12 ± 0.02^{f}	0.97 ± 0.10^{g}	97.65 ± 0.36^{b}	83.69 ± 1.26^{b}
400	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Nystatin 10 µg/mL	3.67 ± 0.16^{c}	2.69 ± 0.16^{e}	27.51 ± 0.98^{e}	54.64 ± 2.03^{d}

The data are expressed as mean ± standard deviation. Different letters in a column indicate a statistically significant difference at $p < 0.05$ (Duncan's test).

Antifungal activities of the CF against blue mold and green mold diseases on orange fruits

The CF of *S. murinus* NARZ significantly inhibited the development of blue and green mold diseases in orange fruits experimentally inoculated with *P. italicum* I6 and *P. digitatum* P2, respectively (**Error! Reference source not found.**). Obvious disease symptoms did not appear in any oranges treated with 100% CF during the experimental period (seven days), and the PIGRs reached 100%. These fruits were firm and fresh. The typical visible symptoms of green and blue mold diseases appeared after five days with various severities on the remaining fruits treated with lower concentrations of CF. At the same concentrations of CF, *P. digitatum* P2 invaded almost all the orange fruits from the wounded sites, while *P. italicum* I6 formed smaller lesion areas after seven days (Figure-4). This meant that the PIGR of CF against blue mold disease was higher than that of green mold disease. At a concentration of 50% CF, the PIGRs reached 49.28% for green mold disease and 84.22% against blue mold disease, respectively. The EC_{50} and MIC_{90} values of CF against green mold

were 47.29% and 90,77%, respectively (y = 0.9199x + 6.5061; R^2 = 0.995). For the blue mold, the EC_{50} and MIC_{90} values of CF were 7.13% and 78.93%, respectively (y = 0.557x + 46.035 and R^2 = 0.9071). The positive control, Fosetyl Aluminum (5 µg/mL), also inhibited the invasion of green and blue mold diseases. The controlled efficacy of Fosetyl Aluminum against green mold disease was similar to that of 40% CF, with 41.34%–42.46% of PIRGs, respectively. However, the fungicide had a higher efficacy on blue mold disease than 40% CF, with 73.32% compared to 62.41%.

Table-6. The PIRG (%) of *S. murinus* NARZ CF against blue mold and green mold disease development on oranges after 7 days

CF concentration, %	PIRG (%)	
	Green mold	Blue mold
10	15.89 ± 1.21^{f}	53.13 ± 2.02^{f}
20	27.75 ± 1.15^{e}	56.75 ± 2.12^{e}
30	33.64 ± 1.23^{d}	58.95 ± 1.53^{e}
40	42.46 ± 1.57^{c}	62.41 ± 1.26^{d}
50	49.28 ± 1.66^{b}	84.22 ± 2.04^{b}
100	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Fosetyl Aluminium (5 µg/mL)	41.34 ± 1.89^{c}	73.32 ± 1.42^{c}

The data are expressed as mean ± standard deviation. Different letters in a column indicate a statistically significant difference at $p < 0.05$ (Duncan's test).

Discussion

In response to growing concerns about public health and environmental well-being, numerous studies worldwide have actively explored novel antibiotics (Qi et al., 2019). In the present study, *S. murinus* NARZ showed the highest antifungal activity against *P. digitatum* P2 and *P. italicum* I6. Interestingly, only one study has reported antifungal activity associated with *S. murinus*, targeting *Pythium aphanidermatum* responsible for damping off disease in watermelons (Ge et al., 2023). Thus, a comprehensive investigation of the antifungal characteristics of this *S. murinus* species is necessary. In this study, we evaluated the antifungal activity of *S. murinus* against *P. digitatum* P2 and *P. italicum* I6 *in vitro* and on orange fruits infected with the pathogens.

The interplay between growth metabolism and the secretion of secondary metabolites in microorganisms is greatly influenced by the composition and concentration of nutrients (Reddy et al., 2011). The disparity observed in the antifungal activities and dried biomass of *S. murinus* NARZ was strongly linked to the constituents of the culture media. Although the International Streptomyces Project medium system was developed to characterize *Streptomyces* species (Shirling and Gottlieb, 1966), each strain has species-specific growth and bioactive metabolite synthesis conditions.

Under nutritionally sufficient conditions, actinomycete cell metabolism rather than secondary metabolites (Bundale et al., 2015; Reddy et al., 2011). Carbon sources also play a key role in this dynamic process.

Figure-4. Efficacy of CF concentrations in inhibiting the growth of blue mold (top row images) and green mold (bottom row images) diseases after seven days

Monosaccharides are known to support biomass development rather than antibiotic production. Conversely, complex carbon sources, such as polysaccharides or slowly utilized nutrients, are believed to stimulate secondary metabolite synthesis (Bundale et al., 2015; Hasani et al., 2014; Jacob et al., 2017). This aligns with our findings, as *S. murinus* NARZ cultivated in ISP3 and ISP4 media containing oatmeal and soluble starch, respectively, exhibited a high PIRG against *Penicillium* pathogens. Both media also facilitated the growth of *S. murinus* NARZ. Interestingly, biomass and antifungal metabolite production of *S. murinus* NARZ were reduced when cultivated in the ISP4G medium. Moreover, adding yeast extract in the ISP4Y medium significantly diminished the antifungal activity of *S. murinus* NARZ. This trend was also observed in the ISP2 medium. The presence of glucose and yeast extract may induce catabolite repression, inhibiting the production of enzymes responsible for secondary metabolite biosynthesis (Reddy et al., 2011). Furthermore, trace minerals, such as zinc, iron, and manganese, in the ISP3 medium could potentially elevate the level of antibiotic production in the *S. murinus* NARZ strain (Hasani et al., 2014).

ISP8 medium, which lacks carbon sources and is rich in nitrogen sources (peptone, meat extract, and KNO3), reduced the production of both antifungal metabolites and biomass in *S. murinus* NARZ. In contrast, potassium nitrate and peptone were deemed favorable nitrogen sources for antifungal production in *S. chilikensis* ACITM-1, followed by yeast and malt extracts (Singh et al., 2017). Similarly, peptone has been demonstrated to be a good source for maximizing the production of bioactive metabolites by *Streptomyces lavendofoliae* Y8 against *Bacillus cereus* (Bundale et al., 2015). Another study revealed that soytone (5%) and maltose (5%) served as the optimal nitrogen sources and carbon for antibiotic synthesis by *S. albidoflavus* C247 (Islam et al., 2009). Considering numerous reports, it is believed that the production of antifungal compounds by a particular strain depends significantly on the composition of the cultivation media and the microorganism used for testing.

The stability under heat conditions is crucial for determining the practicality of bio-preparations (Kaur and Manhas, 2014). Remarkably, the CF from *S. murinus* NARZ demonstrated good thermal tolerance, with PIRGs being well retained when treated at temperatures below 90°C. The PIRGs were only reduced by 25.78% and 35.41% with the CF treated at 121°C for 15 min, for *P. digitatum* P2 and *P. italicum* I6, respectively, in comparison with the PIRG of the CF treated at 30°C for 15 min. Similar findings have been reported for extracts and CF from other *Streptomyces* species. For example, crude extracts from *Streptomyces* sp. TM32 maintained a wide inhibitory zone against *Alternaria alternata* even after autoclaving at 121°C and 15 psi for 15 min (Nakaew et al., 2019). The CF of *S. hydrogenans* DH16 demonstrated thermal stability against *Colletotrichum acutatum* after heating at 37, 50, and 70°C for 1 h. However, the antifungal activity decreased by 10 and 33.33% after boiling the supernatant at 100°C for 1 h and autoclaving (121°C for 30 min), respectively (Kaur and Manhas, 2014). Crude extract of *Streptomyces* sp. H4 was evaluated for its antifungal activity against *Colletotrichum fragariae*, which causes anthracnose in strawberry fruit after heating at 50, 60, 70, 80, 90, 100, and 121°C within 30 min. The antagonistic activity was stable at temperatures below 50°C but became sensitive at temperatures above 60°C, dropping to 38.05% after 121ºC treatment for 30 min (Li et al., 2021). The antifungal activity of *S. murinus* JKTJ-3 against *P. aphanidermatum* was attributed to secondary compounds like actinomycin D and enzymes that break down cell walls, such as chitosanase and β-1,3-glucanase (Ge et al., 2023). The strong thermal resilience of CF from *S. murinus* NARZ in our study suggested that its antifungal properties might be due to antibiotics rather than enzymes or other heat-sensitive substances.

Antifungal activities of *Streptomyces* species have been widely reported on different species and pathogens. Volatile organic compounds produced by *Streptomyces* sp. strain S97, such as monoterpenes, sesquiterpenes, alkenes, alcohols, ketones, pyrazines, and esters, contributed to the inhibitory effect against *Botrytis cinerea* (Ayed et al., 2021). *Streptomyces* sp. SLR03 suppressed the growth of *Pestalotiopsis theae* causing gray blight in tea *in vitro* and *in vivo* because of secondary metabolites, including tetradecane, nonadecane, 10-Henicosene, 3-Eicosene, 1-Hexadecanol, 1-iodo-2-methylundecane, tetradecane, 2,6,10- trimethyl, Decane – 2,3,5,8 trimethyl, Pterin-6-carboxylic acid, and 1-octadecanesulfonylchloride (Marimuthu et al., 2020). *S. cuspidosporus* strain SA4 produced 1,2-Benzenedicarboxylic acid and bis(2-methyl propyl) ester compounds, which demonstrated inhibitory effects against a range of

bacteria and fungi (Sholkamy et al., 2020). On the other hand, the synthesis of extracellular enzymes, such as β-1,3-glucanase and chitinase, has been used to explain for antifungal efficiency of *Streptomyces* species in the previous studies, for example, *S. luomodiensis* against *Fusarium oxysporum* f. sp. *cubense* (Qi et al., 2024), *S. angustmyceticus* against *Lasiodiplodia theobromae* (Ruangwong et al., 2022).

It is noteworthy that previous studies have demonstrated the biological control capabilities of *S. murinus* (Das et al., 2023; Ge et al., 2023). Using MS/MS analysis, Das et al. (2023) reported that actinomycin D, cinnabaramide A, desferrioxamine E, and pentamycin were present in extracts obtained from the supernatant of *S. murinus* THV12. This strain possesses 47 biosynthetic gene clusters responsible for the production of polyketide synthases and non-ribosomal peptide synthetase enzymes, melanin, siderophores, ectoine, lantipeptides, terpenes, lassopeptides, and other putative products (Das et al., 2023). Ge et al. (2023) explained the antifungal mechanism of *S. murinus* strain JKTJ-3 against *P. aphanidermatum* as the production of β-1,3-glucanase, chitinase, and actinomycin D.

In this study, the great inhibitory efficiency of the culture filtrate and ethyl acetate extract thereof, collected from S. murinus NARZ against P. digitatum P2 and P. italicum I6, indicated that it contained antifungal compounds. However, the culture filtrate might contain compounds that did not contribute to antifungal properties, such as some medium residues, or contain unstable antifungal products, like enzymes. Ethyl acetate is a semi-polar solvent that has been widely used to extract unidentified compounds from *Streptomyces* species (Ambarwati et al., 2020; Marimuthu et al., 2020; Nakaew et al., 2019; Sholkamy et al., 2020). Therefore, we aimed to examine if the ethyl acetate crude extract of *S. murinus* shows antifungal activities. The high antifungal effects of the extract obtained from *S. murinus* NARZ against *P. digitatum* P2 and *P. italicum* I6 established a premise for conducting further research that analyzes substances responsible for the antifungal activities of this strain.

The CF of *S. murinus* NARZ not only effectively inhibited the growth of *P. italicum* I6 and *P. digitatum* P2 *in vitro* but also suppressed the development of blue and green mold diseases in orange fruits. The utilization of microbial fermentation broths or their extracts in controlling postharvest diseases has been widely studied. Crude extracts of the *S. chumphonensis* AM-4 strain (1500 mg/L) showed good inhibition against the progression of green mold and blue mold diseases, reaching relative control efficacy of 92,91% and 84,91%, respectively, after five days of inoculation (Hu et al., 2019). Fungal invasion by *P. digitatum* on lemon and orange fruits was reduced after treatment with the ethanolic filtrate of *Streptomyces* sp. 3400 JX826625 (Onja et al., 2022). Culture and metabolite supernatants of *Streptomyces* strain 328 inhibited the development of *P. digitatum* on orange fruits, reducing disease incidence to 22% and 0%, respectively, compared with more than 90% in fruits without treatment with *Streptomyces* strain 328 (Shojaee et al., 2014). Various *Streptomyces* strains have demonstrated good biological control against other postharvest diseases, such as *S. rochei* A-1 against apple ring rot caused by *Botryosphaeria dothidea* (Zhang et al., 2016) and *S. murinus* JKTJ-3 against watermelon damping-off caused by *P. aphanidermatum* (Ge et al., 2023), *S. netropsis* A52M against *Botrytis cinerea* on cherry tomatoes (Montesdeoca-Flores et al., 2023), etc. In this study, 100% CF of *S. murinus* NARZ showed good efficacy in controlling blue and green mold diseases on orange fruits, with 100% PIRGs. The antifungal mechanism of *Streptomyces* species against postharvest fungal pathogens may be due to their potential for secondary metabolite production, including antibiotics, cell wall lytic enzymes, growth promoters, and herbicides (Hu et al., 2019; Khan et al., 2023). Therefore, biological control agents may reduce the use of fungicides in controlling postharvest diseases.

Conclusion

In this study, out of the 48 tested *Streptomyces* strains, *S. murinus* NARZ showed the highest antifungal activity against both *P. digitatum* P2 and *P. italicum* I6. Both CF and CE of *S. murinus* NARZ effectively inhibited the growth of *P. digitatum* P2 and *P. italicum* I6. Of the six tested cultivation media, the CF collected from *S. murinus* NARZ cultured in ISP3 medium showed the highest antifungal activity and good thermal resistance. Furthermore, the CF of *S. murinus* NARZ showed good antifungal efficacy in controlling green mold and blue mold diseases in orange fruits. This is the first study using *S. murinus* isolated from soil to

evaluate biocontrol potential against *P. digitatum* and *P. italicum* pathogens in Vietnam.

Acknowledgment

We thank Prof. Dr. Markus Kalkum of the City of Hope for his assistance with this manuscript.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: This research was funded by the Hue University of Agriculture and Forestry, Hue University, under Project NCM.ĐHNL.2021-04.

Contribution of Authors

Nguyen TTT: Designed the study and wrote the manuscript, Read and approved the manuscript.
Nguyen HH: Performed experiments and collected and analysed data, Read and approved the manuscript.
Le TL: Performed experiments and collected andd analysed data, Read and approved the manuscript.
Nguyen TDH: Performed experiments and collected andd analysed data, Read and approved the manuscript.

References

Abobatta FW, 2019. Nutritional benefits of citrus fruits. Am. J. Biomed. Sci. & Res. 3(4): 303–306. https://doi.org/10.34297/ajbsr.2019.03.000681

Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ, 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410.

Ahmad Z, Abbas H, Murtaza T, Khan AUR, Ali A, Zahid K, Tahir Z, Mahmood T and Habib A, 2023. Assessment of responses of peach cultivars to postharvest pathogen *Botrytis cinerea* and its mitigation using plant essential oils. Plant Protection 7(2):153-162.

Ambarwati A, Wahyuono S, Moeljopawiro S and Yuwono T, 2020. Antimicrobial activity of ethyl acetate extracts of *Streptomyces* sp. CRB46 and the prediction of their bioactive compounds chemical structure. Biodiversitas 21(7):3380-3390 DOI: 10.13057/biodiv/d210763

Ayed A, Kalai-Grami L, Slimene IB, Chaouachi M, Mankai H, Karkouch I, Djebali N, Elkahoui S, Tabbene O and Limam F, 2021. Antifungal activity of volatile organic compounds from *Streptomyces* sp. strain S97 against *Botrytis cinerea*. Biocontrol. Sci. Technol. 31(12): 1330–1348. https://doi.org/10.1080/09583157.2021.1947982

Azish M, Shams-Ghahfarokhi M and Razzaghi-Abyaneh M, 2020. Optimization of the antifungal metabolite production in *Streptomyces libani* isolated from northern forests soils in Iran. Curr. Med. Mycol. 6(4): 20–26. https://doi.org/10.18502/cmm.6.4.5333

Bhatta UK, 2022. Alternative management approaches of citrus diseases caused by *Penicillium digitatum* (Green mold) and *Penicillium italicum* (Blue mold). Front. Plant. Sci. 12: 833328. https://doi.org/10.3389/fpls.2021.833328

Boukaew S, Petlamul W and Prasertsan P, 2020. Comparison of the biocontrol efficacy of culture filtrate from *Streptomyces philanthi* RL-1-178 and acetic acid against *Penicillium digitatum*, in vitro and in vivo. Eur. J. Plant. Pathol. 158(4): 939–949. https://doi.org/10.1007/s10658-020-02128-0

Bundale S, Begde D, Nashikkar N, Kadam T and Upadhyay A, 2015. Optimization of culture conditions for production of bioactive metabolites by *Streptomyces* spp. isolated from soil. Adv. Microbiol. 5(6): 441–451. https://doi.org/10.4236/aim.2015.56045

Chen J, Hu L, Chen N, Jia R, Ma Q and Wang Y, 2021. The biocontrol and plant growth-promoting properties of *Streptomyces alfalfae* XN-04 revealed by functional and genomic analysis. Front. Microbiol. 12: 745766. https://doi.org/10.3389/fmicb.2021.745766

Das V, Chatterjee NS, Pushpakaran PU, Lalitha KV and Joseph TC, 2023. Exploration of natural product repository by combined genomics and metabolomics profiling of mangrove-derived *Streptomyces murinus* THV12 strain. Fermentation. 9(6): 576. https://doi.org/10.3390/fermentation9060576

Dezfully NK and Ramanayaka JG, 2015. Isolation, identification and evaluation of antimicrobial activity of *Streptomyces flavogriseus*, strain ACTK2 from soil sample of Kodagu, Karnataka State (India). Jundishapur. J. Microbiol. 8(2): e15107. https://doi.org/10.5812/jjm.15107

El-majberi Saleh HM, Alraaydi Nagwa SA and Elyass ME, 2020. Isolation and identification of green mould *Penicillium* species on harvested

citrus fruit. AJRBPS. 8(3): 120–127. DOI 10.36673/AJRBPS.2020.v08.i03.A15.

Freimoser FM, Rueda-Mejia MP, Tilocca B and Migheli Q, 2019. Biocontrol yeasts: mechanisms and applications. World J. Microbiol. Biotechnol. 35(10):154. doi: 10.1007/s11274-019-2728-4.

Ge M, Cai X, Wang D, Liang H, Zhu J, Li G and Shi X, 2023. Efficacy of *Streptomyces murinus* JKTJ-3 in suppression of *Pythium* damping-off of watermelon. Microorganisms. 11(6): 1360. https://doi.org/10.3390/microorganisms11061360

Hasani A, Kariminik A and Issazadeh K, 2014. Streptomycetes: Characteristics and their antimicrobial activities. Int. J. Adv. Biol. Biomed. Res. 2(1): 63–75.

Hu X, Cheng B, Du D, Huang Z, Pu Z, Chen G, Peng A and Lu L, 2019. Isolation and identification of a marine actinomycete strain and its control efficacy against citrus green and blue moulds. Biotechnol. Biotechnol. Equip. 33(1): 719–729. https://doi.org/10.1080/13102818.2019.1613175

Islam MR, Jeong YT, Ryu YJ, Song CH and Lee YS, 2009. Isolation, identification and optimal culture conditions of *Streptomyces albidoflavus* C247 producing antifungal agents against *Rhizoctonia solani* AG2-2. Mycobiology. 37(2): 114-20. https://doi.org/10.4489/myco.2009.37.2.114

Jacob J, Rajendran RU, Priya SH, Purushothaman J and Amma DKBNS, 2017. Enhanced antibacterial metabolite production through the application of statistical methodologies by a *Streptomyces nogalater* NIIST A30 isolated from Western Ghats forest soil. PLoS ONE. 12(4): 1–21. https://doi.org/10.1371/journal.pone.0175919

Kaur T and Manhas RK, 2014. Antifungal, insecticidal, and plant growth promoting potential of *Streptomyces hydrogenans* DH16. J. Basic. Microbiol. 54(11): 1175–1185. https://doi.org/10.1002/jobm.201300086

Khan S, Srivastava S, Karnwal A and Malik T, 2023. *Streptomyces* as a promising biological control agents for plant pathogens. Front. Microbiol. 14: 1285543. https://doi.org/10.3389/fmicb.2023.1285543

Ladaniya MS, 2008. Citrus Fruit: Biology, Technology and Evaluation. Elsevier Inc., Atlanta, USA, 1-10. http://dx.doi.org/10.1016/B978-012374130-1.50003-6

Li X, Jing T, Zhou D, Zhang M, Qi D, Zang X, Zhao Y, Li K, Tang W, Chen Y, Qi C, Wang W and Xie J, 2021. Biocontrol efficacy and possible mechanism of *Streptomyces* sp. H4 against postharvest anthracnose caused by *Colletotrichum fragariae* on strawberry fruit. Postharvest. Biol. Technol. 175: 111401. https://doi.org/10.1016/j.postharvbio.2020.111401

Marimuthu S, Karthic C, Mostafa AA, Mohammed Al-Enazi N, Abdel-Raouf N and Sholkamy EN, 2020. Antifungal activity of *Streptomyces* sp. SLR03 against tea fungal plant pathogen *Pestalotiopsis theae*. J. King. Saud. Univ. Sci. 32(8): 3258–3264. https://doi.org/10.1016/j.jksus.2020.08.027

Montesdeoca-Flores DT, Hernández-Bolaños E, León-Barrios M, Hernández-Amador E, Díaz-González S, Abreu-Acosta N and Luis-Jorge JC, 2023. Antifungal activity of *Streptomyces* spp. extracts in vitro and on post-harvest tomato fruits against plant pathogenic fungi. Horticulturae, 9(12). https://doi.org/10.3390/horticulturae9121319

Nakaew N, Lumyong S, Sloan WT and Sungthong R, 2019. Bioactivities and genome insights of a thermotolerant antibiotics-producing *Streptomyces* sp. TM32 reveal its potentials for novel drug discovery. MicrobiologyOpen. 8: e842. https://doi.org/10.1002/mbo3.842

Nguyen TTT, Nguyen HT, Nguyen TDH and Le TL, 2022. Evaluation antifungal ability of *Streptomyces* sp. against *Collectotrichum* causing anthracnose on dragon fruit. Vietnam Journal of Agricultural Sciences. 20(12): 1591–1598. (In Vietnamese with English abstract)

Noman E, Al-Gheethi AA, Rahman NK, Talip B, Mohamed R, H N and Kadir OA, 2018. Single spore isolation as a simple and efficient technique to obtain fungal pure culture, pp. 1 – 5. In Proceedings, Conf. Ser.: Earth Environ. Sci., 4th International Conference on Civil and Environmental Engineering for Sustainability (IConCEES 2017)4–5 December 2017, Langkawi, Malaysia.

Onja A, Rigobert A, Eddy AM, Luciano R and Rado R, 2022. Antifungal potential of *Streptomyces* sp. 3400 JX826625 ethanolic filtrate against *Penicillium digitatum*, a post-harvest spoilage agent of citrus fruits. Int. J. Micro. Myco. 15(4): 11-25.

Palou L, 2014. *Penicillium digitatum*, *Penicillium italicum* (Green Mold, Blue Mold), pp. 45–102.

In S. Bautista-Baños (ed), Postharvest Decay: Control Strategies. Elsevier Inc.

Perez MF, Díaz MA, Pereyra MM, Córdoba JM, Isas AS, Sepúlveda M, Ramallo J and Dib JR, 2019. Biocontrol features of *Clavispora lusitaniae* against *Penicillium digitatum* on lemons. Postharvest. Biol. Technol. 155: 57–64. https://doi.org/10.1016/j.postharvbio.2019.05.012

Pitt JI and Hocking AD, 2009. Fungi and Food Spoilage, 3rd ed. Springer Dordrecht Heidelberg London New York Cambridge.

Procópio RE, Silva IR, Martins MK, Azevedo JL and Araújo JM, 2012. Antibiotics produced by *Streptomyces*. Braz. J. Infect. Dis. 16(5): 466–471. https://doi.org/10.1016/j.bjid.2012.08.014

Qi D, Liu Q, Zou L, Zhang M, Li K, Zhao Y, Chen Y, Feng J, Zhou D, Wei Y, Wang W, Zhang L and Xie J, 2024. Taxonomic identification and antagonistic activity of *Streptomyces luomodiensis* sp. nov. against phytopathogenic fungi. Front. Microbiol. 1. https://doi.org/10.3389/fmicb.2024.1402653

Qi D, Zou L, Zhou D, Chen Y, Gao Z, Feng R, Zhang M, Li K, Xie J and Wang W, 2019. Taxonomy and broad-spectrum antifungal activity of *Streptomyces* sp. SCA3-4 isolated from rhizosphere soil of opuntia stricta. Front. microbiol. 10: 1390. https://doi.org/10.3389/fmicb.2019.01390

Reddy N, Ramakrishna D and Rajagopal S, 2011. Optimization of culture conditions of *Streptomyces rochei* (MTCC 10109) for the production of antimicrobial metabolites. Egypt. J. Biol. 13(1): 21–29. https://doi.org/10.4314/ejb.v13i1.4

Ruangwong OU, Kunasakdakul K, Daengsuwan W, Wonglom P, Pitija K and Sunpapao A, 2022. A Streptomyces rhizobacterium with antifungal properties against spadix rot in flamingo flowers. Physiol. Mol. Plant Pathol. 117, 101784. https://doi.org/https://doi.org/10.1016/j.pmpp.2021.101784

Shirling EB and Gottlieb D, 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16(3): 313–340. https://doi.org/http://dx.doi.org/10.1099/00207713-16-3-313

Shojaee N, Shahidi BGH, Shahdaei S and Leyla SB, 2014. Biological control of citrus green mould, *Penicillium digitatum*, by antifungal activities of *Streptomyces* isolates from agricultural soils. Afr. J. Microbiol. Res. 8(14): 1501–1509. https://doi.org/10.5897/ajmr2013.6289

Sholkamy EN, Muthukrishnan P, Abdel-Raouf N, Nandhini X, Ibraheem IBM and Mostafa AA, 2020. Antimicrobial and antinematicidal metabolites from *Streptomyces cuspidosporus* strain SA4 against selected pathogenic bacteria, fungi and nematode. Saudi. J. Biol. Sci. 27(12): 3208–3220. https://doi.org/10.1016/j.sjbs.2020.08.043

Singh C, Singh PR, Jadon P and Kumar A, 2017. Optimization of cultural conditions for production of antifungal bioactive metabolites by *Streptomyces* spp. isolated from soil. Int. J. Curr. Microbiol. Appl. Sci. 6(2): 386–396. https://doi.org/10.20546/ijcmas.2017.602.043

Tamura K, Stecher G and Kumar S, 2021. MEGA11: Molecular evolutionary genetics analysis version 11. MBE 38:3022–3027.

Wang Z, Jiang M, Chen K, Wang K, Du M, Zalán Z, Hegyi F and Kan J, 2018. Biocontrol of *Penicillium digitatum* on postharvest citrus fruits by *Pseudomonas fluorescens*. Journal of Food Quality. 1–9. https://doi.org/10.1155/2018/2910481

Wang Z, Sui Y, Li J, Tian X and Wang Q, 2022. Biological control of postharvest fungal decays in citrus: a review. Crit. Rev. Food. Sci. Nutr. 62(4): 861–870. https://doi.org/10.1080/10408398.2020.1829542

Yong D, Li Y, Gong K, Yu Y, Zhao S, Duan Q, Ren C, Li A, Fu J, Ni J, Zhang Y and Li R, 2022. Biocontrol of strawberry gray mold caused by *Botrytis cinerea* with the termite associated *Streptomyces* sp. sdu1201 and actinomycin D. Front. Microbiol. 13: 1051730. https://doi.org/10.3389/fmicb.2022.1051730

Zarrin M, Ganj F and Faramarzi S, 2016. Analysis of the rDNA internal transcribed spacer region of the fusarium species by polymerase chain reaction restriction fragment length polymorphism. Biomed. Rep. 4(4):471–474. https://doi.org/10.3892/br.2016.615

Zhang Q, Yong D, Zhang Y, Shi X, Li B, Li G, Liang W and Wang C, 2016. *Streptomyces rochei* A-1 induces resistance and defense-related responses against *Botryosphaeria dothidea* in apple fruit during storage. Postharvest. Biol. Technol. 115: 30–37. https://doi.org/10.1016/j.postharvbio.2015.12.013