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Endophytic Fungi Associated with Mango Show In Vitro Antagonism against Bacterial and Fungal Pathogens

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Abstract: Endophytic fungi live in inter-cellular spaces of healthy plant tissues without causing any apparent symptoms of diseases for the host plant. Some fungal endophytes help their plant hosts to survive under biotic and abiotic stresses. In this study, we collected healthy mango leaves at the Honghe mango plantations (Yunnan Province) in the winter. A total of 34 different fungal endophytic strains were isolated, and their phylogenetic placements were estimated based on the ITS gene. Members of genus *Chaetomium* were the dominant fungal endophytes (26%). Common bacterial plant pathogens (*Erwinia amylovora* and *Pseudomonas syringae*) and fungal plant pathogens (*Botrytis cinerea* and *Penicillium digitatum*) were selected to test the antagonism of the fungal endophytes isolated from mango leaves through co-cultivation in vitro assay. Three strains of *Chaetomium* sp. viz. KUNCC22-0749, UNCC22-10750, and KUNCC22-10752 showed great inhibition against two bacterial pathogens viz. *Erwinia amylovora* and *Pseudomonas syringae*, and *Alternaria* sp. KUNCC22-10760, *Chaetomium* sp. KUNCC22-10749, *Daldinia* sp. KUNCC22-10744, and *Rosellinia* sp. KUNCC22-10751 also showed great to moderate antagonistic effects against two fungal pathogens viz. *Botrytis cinerea* and *Penicillium digitatum*.

Keywords: antagonistic activities; *Chaetomium*; fungal endophytes; mango; Yunnan Province

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1. Introduction

Phytopathogenic fungi are among the dominant agents of plant diseases that result in enormous losses in yield and quality of field crops, fruits, and other edible plant materials [1]. Based on the mode of nutrition, phytopathogenic fungi are normally classified into two major groups: biotrophic and necrotrophic pathogens. Biotrophic pathogens (biotrophs) have close relationships with their hosts and are able to use living tissues to obtain nutrients, while necrotrophic pathogens (necrotrophs) kill plant tissues and obtain nutrients [2]. Fungal infections cause a wide variety of disease symptoms [3]. Green mold and associated decay caused by *Penicillium digitatum* is the most devastating disease in postharvest citrus fruits (oranges, tangerines, lemons, and grapefruit). About 90% of

postharvest losses in citriculture have been observed to be caused by *P. digitatum* in arid regions and tropical subclimates [4,5]. In addition, *Botrytis cinerea* (teleomorph *Botryotinia fuckeliana*) has been reported to attack mainly more than 200 dicotyledonous plants, especially as the causal agent of grey mold or botrytis bunch rot in vineyards, causing serious economic losses worldwide [6–8].

Phytopathogenic bacteria are important plant pathogens widespread throughout the world [9]. Rajesh-Kannan et al. [10] estimated that about 150 bacterial species are responsible for different plant diseases, and they mainly belong to three families: *Enterobacteriaceae*, *Pseudomonaceae*, and *Xantomonadaceae. Erwinia amylovora* (*Enterobacteriaceae*), a causal agent of fire blight on a variety of host species in the Rosaceae, causes severe hazards to the production of *Malus*, *Pyracantha*, *Pyrus*, and *Rubus* [11–13]. Moreover, *Pseudomonas syringae* (*Pseudomonaceae*) includes 15 identified species and more than 60 pathovars [14]. Almost all *Pseudomonas syringae* pathovars are able to infect over 180 plant species and are exceedingly difficult to control, especially since they result in bacterial cankers on various economically significant fruits crops from genera *Actinidia*, *Mangifera*, and *Prunus* [15–18]. *Pseudomonas syringae* pv. *syringae* is known as the most polyphagous bacterium that has a broad host range [17,19]. Mansfield et al. [20] mentioned that both *E. amylovora* and *P. syringae* are listed among the top 10 plant pathogenic bacteria.

The word "endophyte" is derived from Greek, meaning inside or within plants [21]. Endophytic fungi live entirely within plant tissues without causing any apparent symptoms of diseases and emerge to sporulate at the plant or host-tissue senescence [22,23]. Several factors influence biological characteristics of endophytes, such as host species, host developmental stage, inoculum density, and environmental conditions, and they play a significant role in the control of plant pathogen communities [24]. Endophytic fungi help plant hosts survive under biotic and abiotic stresses, and considerable evidence has shown that some endophytic fungi have the ability to protect host plants from attacks from pathogens and insects [25] and environmental stresses [26,27]. However, the delicate relationships between most fungal endophytes and their plant hosts have still not been well understood [28,29]. In addition, the secondary metabolites produced by endophytic fungi appear to have potential as anticancer, insecticidal, antidiabetic, immunosuppressive, and biocontrol agents. Therefore, the intensive studies of endophytic fungi will be helpful in the industrial, pharmaceutical, medical, and agricultural sectors [30].

Mango-associated fungal endophytes have been poorly studied. Vieira et al. [31] isolated 22 fungal endophytic strains of *Colletotrichum* associated with mango in South China. Dashyal et al. [32] isolated 35 strains of endophytic fungi from the stem and leaves of 10 mango varieties. In recent years, endophytic fungi have been regarded as exciting novel sources of new bioactive compounds, with reports from a variety of hosts [33–35]. Nwakanma et al. [36] reported that secondary metabolites of endophytic fungi isolated from bush mango leaves have antimicrobial activities. Phytopathogenic bacteria and fungi are severe on economic crops, and chemical treatments are the most used control strategies [1,9,37,38]. However, as a result of the widespread and repeated use of certain chemical fungicides, a number of pathogenic strains have become fungicide-resistant, and fungicide residues have caused environmental pollution and harmed soil and water animals [39]. Endophytic fungi are eco-friendly and effective biocontrol agents against various bacterial and fungal pathogens [40,41], while endophytic fungi associated with mango are poorly studied. Therefore, investigating the diversity of endophytic fungi associated with mango and screening the antagonistic strains are useful for controlling fungal and bacterial pathogens in the field. The aims of this study were to investigate the endophytic fungi associated with mango and screen endophytic strains with biocontrol potentials.

2. Materials and Methods

2.1. Sampling Mango Leaves and Endophytes Isolation

Fresh and healthy mango leaves were picked from well-managed trees in the Honghe prefecture, Yunnan Province, in December 2020 (Figure 1). The local GPS and elevation

information (102°50′11″ E, 23°41′01″ N, 500 ± m) was recorded, and 100 leaves were picked from different mango trees and transported to the mycology laboratory in disposable sterilized bags. Tibpromma et al. [42] was followed for the isolation of endophytic fungi. The collected leaf samples were washed with tap water, cut into small pieces, and surface sterilized in sodium hypochlorite (3%) for 1 min, followed by washing in sterilized water and 75% ethanol for 1 min. Finally, they were washed in sterile distilled water 3 times and dried in sterilized tissue papers. Four sterilized leaf pieces were inoculated to a potato dextrose agar (PDA) medium and incubated at 27 °C for 1–2 days. Once the hypha emerges from leaf tissues, the tips were picked up to new PDA plates [43]. The morphology of colonies on PDA was taken with a camera of a Huawei P40 mobile phone (Huawei, Shenzhen, China). Fungal cultures were deposited in the Kunming Institute of Botany Culture Collection, China (KUNCC).

Figure 1. (**a**,**b**) Mango fruits and flowers with healthy leaves in Honghe, Yunnan Province, China.

2.2. DNA Extraction, PCR Amplification and Sequencing

After the fungi had grown for around a week on PDA plates, those cultures were used for DNA extraction. The fresh mycelia (30–50 mg) were scraped from pure fungal colonies and transferred into 1.5 mL sterilized microcentrifuge tubes. Genomic DNA was extracted by the Biospin Fungus Genomic DNA Extraction Kit–BSC14S1 (BioFlux®, Hangzhou, China), following the manufacturer's guidelines. A part of extracted DNA was stored at 4 °C for the instant PCR amplification, and the remaining portion was kept at −20 °C for long-term storage. PCR mixture contained 12.5 µL of 2× Power Taq PCR MasterMix (mixture of EasyTaqTM DNA Polymerase, dNTPs, 8.5 µL of double-distilled water (ddH_2O), optimized buffer (Beijing Bio Teke Corporation (Bio Teke), Beijing, China [44]) and 1 µL of each forward and reverse primers (10 pmol), and 2 µL of DNA templet. Using the primers ITS4/ITS5, the internal transcribed spacer (ITS) region was amplified [45]. The PCR condition of ITS genes constituted an initial denaturation step of 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 50 s at 55 °C, 1 min at 72 °C, and a final denaturation step of 10 min at 72 °C. The PCR products were purified and sequenced at Beijing Bio Teke Corporation.

2.3. Sequence Alignment and Phylogenetic Analyses

The reverse and forward sequences were checked in BioEdit v. 7.0.9.0 [46] and assembled in the Geneious (Restricted) 9.1.2 (website: https://www.geneious.com, accessed on 20 May 2022). Each sequence was BLASTn searched in the GenBank (website: http://blast.ncbi.nlm.nih.gov/, accessed on 20 May 2022) to screen the taxa with the highest degree of similarity. The ITS sequence alignment was made in the MAFFT online server (website: www.ebi.ac.uk/Tools/mafft, accessed on 20 May 2022) [47] and minor alterations in BioEdit 7.2.3 [46], whenever necessary. TrimAL v1.2 (website: http://trimal.cgenomics.org,

accessed on 20 March 2022) was used to eliminate the uninformative gaps and unclear regions in the data alignment. On the CIPRES Science Gateway v.3.3, maximum likelihood analysis (ML) was performed (website: http://www.phylo.org/portal2, accessed on 20 May 2022 [48]), selecting RAxML-HPC2 on XSEDE (8.2.12) [49] with the GTRGAMMA substitution model with 1000 bootstrap iterations. FigTree v1.4.0 was used to display phylogenetic trees [50], while Microsoft PowerPoint (Microsoft Inc., Redmond, WA, USA) was used to edit the tree and reliable bootstrap support values were inserted from ML. Newly generated sequences were deposited in GenBank.

2.4. Screening Antagonistic Endophytes by Dual Culture Assay

For screening antagonistic fungal endophytes, two bacterial (*Pseudomonas syringae* and *Erwinia amylovora*) and two fungal (*Penicillium digitatum* and *Botrytis cinerea*) plant pathogens were obtained from China General Microbiological Culture Collection Center (CGMCC) (Table 1). The 34 fungal endophytes and four phytopathogenic species were respectively inoculated in the media (fungi: potato dextrose agar (PDA), bacteria: nutrient agar (NA)) for 10 days at 27 °C to make sure all strains have the same growing age [51–54]. At 10 days, the mycelium discs of 3.0 mm diameter from both the endophytes and the phytopathogenic fungi were taken out, and the plugs were co-inoculated equidistantly in 85 mm PDA Petri dishes spaced 10 mm from the edge of the dish, while 3.0 mm width of phytopathogenic bacteria were streaked in the opposite of fungal endophytes in NA Petri dishes. All endophyte-phytopathogen antagonism tests were performed in triplicate, and the plates (triplicate) that were only inoculated with bacterial and fungal pathogens were used as the control groups. After incubating at 27 °C for 10 days, the radial growth of bacterial/fungal pathogens was measured, provided the endophytic fungi overrun the pathogens, and the measured value was obtained from the reverse side. The antagonistic property of each endophyte was expressed as percentage inhibition of radial growth of fungal pathogens and width of the bacterial streak (PIRG-P), using the formula PIRG-P(%) = [(R1 − R2)/R1] × 100%, where: PIRG-P = Percentage inhibition; R1 = The radial growth of the fungal pathogens in control plates/The width of bacterial streak; R2 = The radial growth of the fungal pathogens/The width of bacterial streak at 10 days of antagonism trials [51–54].

Table 1. Bacterial and fungal pathogens from China General Microbiological Culture Collection Center (CGMCC).

	Species	**Strain**	**References**
Bacterial pathogens	*Pseudomonas syringae*	CGMCC: 1.3333	[55]
	Erwinia amylovora	CGMCC: 1.7276	[20]
Fungal pathogens	*Penicillium digitatum*	CGMCC: 3.15410	[5]
	Botrytis cinerea	CGMCC: 3.3790	[7]

2.5. Statistical Analysis

The inhibition rate was analyzed using IBM-SPSS (Statistic Product and Service Solutions) Statistics for Windows, version 29. 0. (SPSS Inc., Chicago, IL, USA). Data were analyzed by the one-way ANOVA with LSD and Duncan tests at the significant level $p < 0.05$. All values were expressed as means of three replicates ± standard deviation (S.D.). The visual bar chart was formed in GraphPad Prism software version 9.0 (GraphPad Holdings, San Diego, CA, USA) statistical package.

3. Results

3.1. Diversity of Endophytic Fungi and Phylogenetic Analyses Based on the ITS Gene

In this study, we isolated 34 fungal endophytic strains, and their phylogenetic placements were given based on the ITS locus. The colony morphology in the PDA of each strain was exhibited beside ML (Maximum likelihood analysis) tree (Figure 2). The results show that the 34 strains belong to 3 different classes (*Dothideomycetes*, *Pezizomycetes*,

and *Sordariomycetes*); 12 different orders (*Amphisphaeriales*, *Botryosphaeriales*, *Calosphaeriales*, *Capnodiales*, *Diaporthales*, *Glomerellales*, *Hypocreales*, *Mycosphaerellales*, *Pezizales*, *Pleosporales*, *Sordariales*, and *Xylariales*); and 20 different families. In addition, *Chaetomiaceae* (*Chaetomium* spp.) isolates showed the highest diversity, which accounts for 26% (9 strains) among all the isolates (Figure 3).

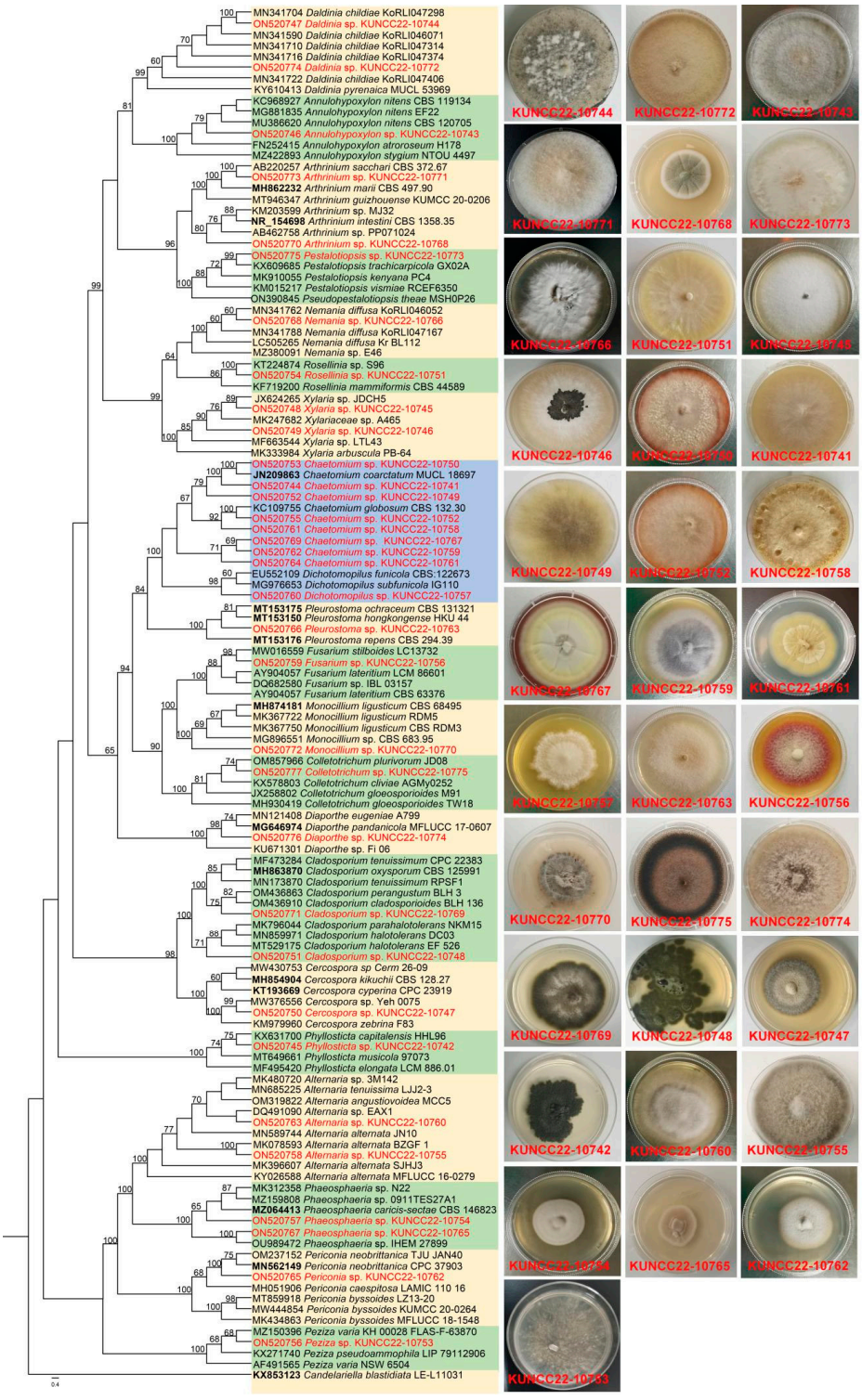

Figure 2. The left side shows the phylogram generated from maximum likelihood analysis based on an ITS sequence dataset. The tree is rooted with *Candelariella blastidiata* (LE-L11031). The ML

bootstrap support values equal to or greater than 60% are shown at the nodes. Type strains are indicated in bold. The right-side images show all the endophytic fungi cultures that were grown on PDA at room temperature for two weeks and their culture collection numbers are written at the bottom of the culture image.

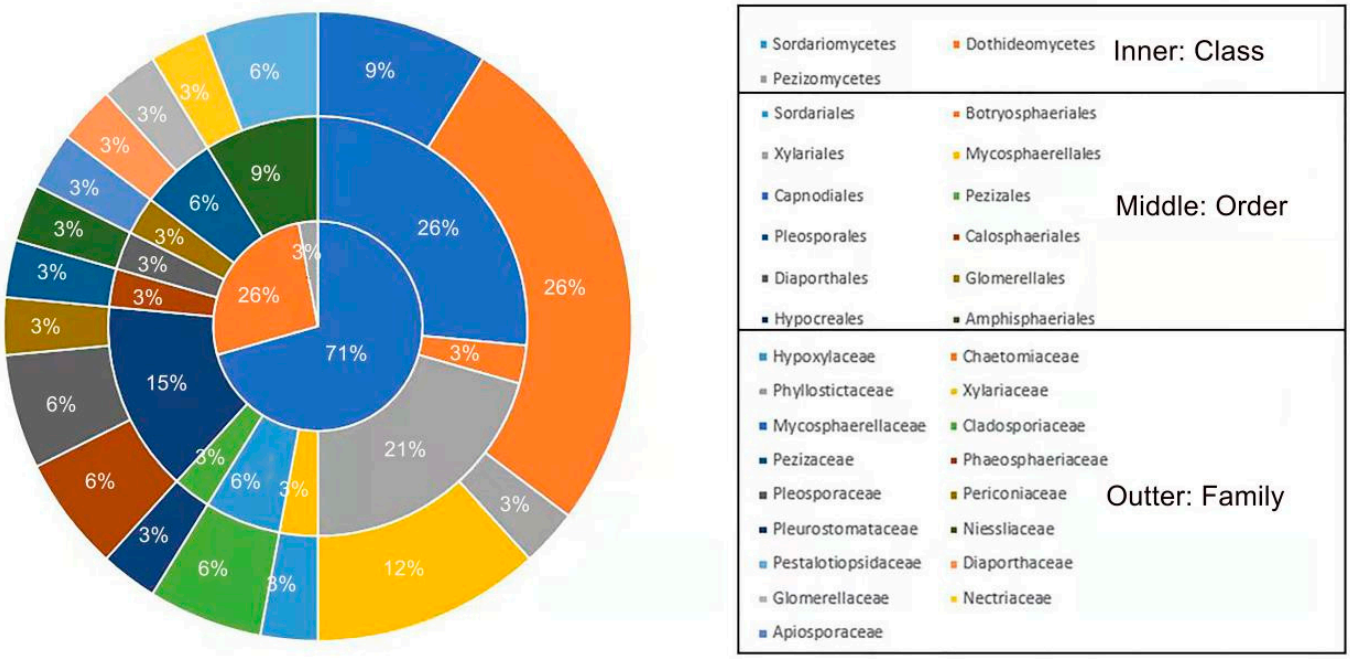

Figure 3. Classification of endophytic fungi (n = 34) associated with mango leaves.

3.2. In Vitro Biocontrol Experiments

3.2.1. Effect of Endophytes on the Growth of *Erwinia amylovora* (CGMCC: 1.7276)

Colonies of *Erwinia amylovora* were observed as white colonies in NA media. *Chaetomium* sp. KUNCC22-10749 strain inhibited the growth of *E. amylovora* by forming a fast-growing plentiful aerial mycelium within a short time (Figure 4b), at an inhibition rate of 50.00 ± 1.63% (Table 2). *Chaetomium* sp. KUNCC22-10750 strain inhibited the growth of *E. amylovora* by forming a clear zone between the fungus and the bacterium (Figure 4c) at an inhibition rate of 58.49 ± 3.27% (Table 2).

3.2.2. Effect of Endophytes on the Growth of *Pseudomonas syringae* (CGMCC: 1.3333)

Pseudomonas syringae was visible as orange-yellow, slimy colonies in NA media. *Chaetomium* sp. KUNCC22-10752 strain inhibited the growth of *P. syringae* (Figure 4e) with an inhibition rate of 50.67 ± 2.00% (Table 2). The pathogenic strain became dry, stopped growing, and covered with the mycelium of fungal endophytes.

Table 2. The radial growth inhibition rates of fungal and bacterial pathogens. Inhibitions equal to or greater than 50% are in black bold.

Collection No.	Genera	GenBank Accession Number (ITS)	Growth Inhibition Rate (GI) of 10 Days ± SD (%)			
			Erwinia amylovora CGMCC: 1.7276	*Pseudomonas syringae* CGMCC 1.3333	*Botrytis cinerea* CGMCC: 3.3790	*Penicillium digitatum* CGMCC: 3.15410
KUNCC22-10741	*Chaetomium*	ON520744	18.86 ± 3.27 c–g	16.00 ± 4 f–i	31.54 ± 5.06 l–n	17.75 ± 2.65 h–l
KUNCC22-10742	*Phyllosticta*	ON520745	13.20 ± 6.54 e–j	8.00 ± 4 j–m	39.82 ± 4.17 d–l	31.34 ± 2.11 c–f
KUNCC22-10743	*Annulohypoxylon*	ON520746	22.64 ± 3.27 c–e	13.33 ± 2.31 g–j	37.61 ± 5.82 f–m	29.96 ± 6.39 c–g
KUNCC22-10744	*Daldinia*	ON520747	7.54 ± 3.27 h–j	2.67 ± 2.31 lm	**55.56 ± 1.73 b**	**51.16 ± 5.59 b**
KUNCC22-10745	*Xylaria*	ON520748	7.54 ± 1.63 h–j	18.67 ± 2.31 fg	29.88 ± 2.53 mn	28.58 ± 2.11 c–h
KUNCC22-10746	*Xylaria*	ON520749	11.32 ± 8.65 f–j	21.33 ± 2.31 ef	40.92 ± 4.17 d–j	23.05 ± 8.10 e–j
KUNCC22-10747	*Cercospora*	ON520750	6.60 ± 2.83 ij	10.67 ± 4.62 h–k	32.92 ± 3.79 j–n	25.81 ± 0.80 d–i
KUNCC22-10748	*Cladosporium*	ON520751	13.20 ± 8.65 e–j	30.67 ± 2.31 b–d	33.20 ± 0.96 i–n	20.29 ± 2.88 f–k
KUNCC22-10749	*Chaetomium*	ON520752	**50.00 ± 1.63 b**	29.33 ± 2.31 cd	**52.02 ± 2.92 bc**	38.25 ± 6.39 c
KUNCC22-10750	*Chaetomium*	ON520753	**58.49 ± 3.27 a**	49.33 ± 2.31 a	42.58 ± 2.66 d–h	34.57 ± 4.23 cd
KUNCC22-10751	*Rosellinia*	ON520754	9.43 ± 5.66 g–j	2.00 ± 2.11 m	47.55 ± 4.17 cd	**60.83 ± 2.88 a**
KUNCC22-10752	*Chaetomium*	ON520755	45.29 ± 3.27 b	**50.67 ± 2.00 a**	32.09 ± 4.97 k–n	28.12 ± 2.77 c–h
KUNCC22-10753	*Peziza*	ON520756	24.53 ± 3.27 cd	18.67 ± 6.11 fg	44.24 ± 3.45 c–g	6.00 ± 2.77 m
KUNCC22-10754	*Phaeosphaeria*	ON520757	11.32 ± 8.65 f–j	12.00 ± 4.00 g–k	43.13 ± 1.26 d–h	27.66 ± 2.11 c–i
KUNCC22-10755	*Alternaria*	ON520758	26.42 ± 5.67 c	36.00 ± 4.00 bc	40.37 ± 4.39 d–k	17.06 ± 2.77 i–l
KUNCC22-10756	*Fusarium*	ON520759	24.53 ± 3.27 cd	17.33 ± 2.31 f–h	39.27 ± 1.26 d–l	16.05 ± 1.72 i–l
KUNCC22-10757	*Chaetomium*	ON520760	18.87 ± 11.79 c–g	7.33 ± 4.16 j–m	32.09 ± 6.63 k–n	33.65 ± 9.97 c–e
KUNCC22-10758	*Chaetomium*	ON520761	22.64 ± 3.27 c–e	22.67 ± 6.11 ef	36.51 ± 6.70 g–m	28.11 ± 2.4 c–h
KUNCC22-10759	*Chaetomium*	ON520762	16.98 ± 6.54 c–h	10.67 ± 2.31 h–k	45.34 ± 3.31 c–f	30.42 ± 9.81 c–g
KUNCC22-10760	*Alternaria*	ON520763	20.75 ± 5.67 c–f	32.00 ± 4.00 b–d	**67.15 ± 5.88 a**	29.96 ± 9.71 c–g
KUNCC22-10761	*Chaetomium*	ON520764	16.98 ± 3.27 c–h	9.33 ± 2.31 i–l	29.88 ± 2.53 mn	20.74 ± 1.6 f–k
KUNCC22-10762	*Periconia*	ON520765	9.43 ± 0.00 g–j	26.67 ± 2.31 de	40.37 ± 1.66 d–k	37.8 ± 3.66 c
KUNCC22-10763	*Pleurostoma*	ON520766	5.66 ± 3.27 ij	21.33 ± 4.62 ef	41.75 ± 4.25 d–i	34.11 ± 6.54 cd
KUNCC22-10765	*Phaeosphaeria*	ON520767	4.71 ± 1.63 j	17.33 ± 2.31 f–h	42.86 ± 4.61 d–h	10.60 ± 8.45 k–m
KUNCC22-10766	*Nemania*	ON520768	19.81 ± 5.9 c–f	6.00 ± 2.00 k–m	27.12 ± 4.38 n	21.67 ± 8.89 f–j
KUNCC22-10767	*Chaetomium*	ON520769	18.87 ± 6.54 c–g	32.00 ± 4.00 b–d	26.57 ± 5.06 n	23.97 ± 4.99 d–j
KUNCC22-10768	*Arthrinium*	ON520770	14.15 ± 4.32 e–j	36.67 ± 7.57 b	34.58 ± 8.41 h–n	31.34 ± 2.88 c–f
KUNCC22-10769	*Cladosporium*	ON520771	22.64 ± 3.27 c–e	31.33 ± 3.06 b–d	37.61 ± 3.45 f–m	19.82 ± 2.77 g–k
KUNCC22-10770	*Monocillium*	ON520772	7.54 ± 3.27 h–j	6.00 ± 5.29 k–m	38.72 ± 3.32 e–l	9.68 ± 4.22 lm
KUNCC22-10771	*Arthrinium*	ON520773	13.2 ± 3.27 e–j	3.33 ± 1.15 lm	39.54 ± 0.83 d–l	21.67 ± 1.60 f–j
KUNCC22-10772	*Daldinia*	ON520774	4.71 ± 1.63 j	10.67 ± 6.11 h–k	46.45 ± 8.17 c–e	21.66 ± 11.51 f–j
KUNCC22-10773	*Pestalotiopsis*	ON520775	15.09 ± 5.66 d–i	21.33 ± 2.31 ef	29.88 ± 2.53 mn	28.40 ± 8.16 c–h
KUNCC22-10774	*Diaporthe*	ON520776	20.75 ± 5.67 c–f	9.33 ± 2.31 i–l	42.31 ± 0.48 d–h	25.81 ± 2.11 d–i
KUNCC22-10775	*Colletotrichum*	ON520777	22.64 ± 3.27 c–e	2.67 ± 2.31 lm	31.54 ± 6.69 l–n	14.30 ± 3.91 j–m

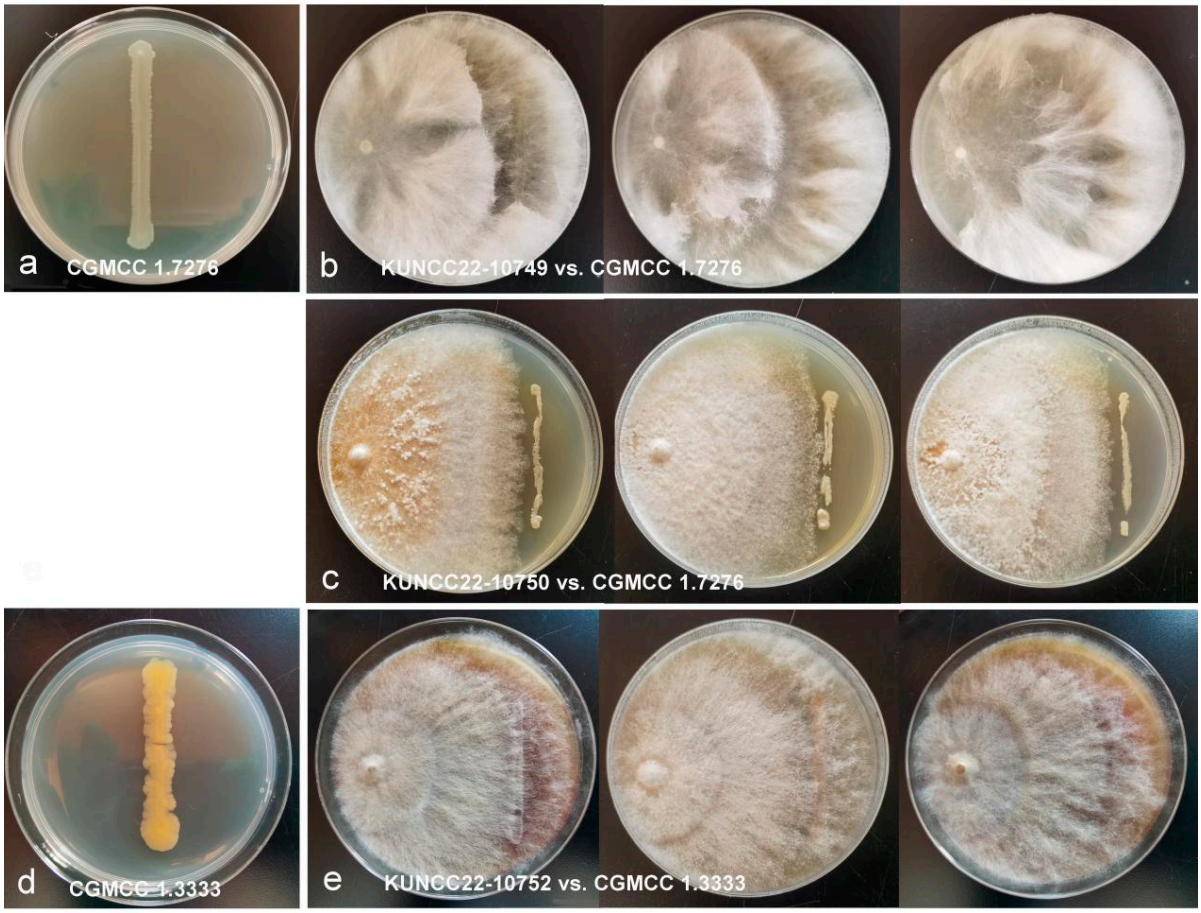

Figure 4. The images of two bacterial pathogens that were co-cultivated with the mango fungal endophytes on NA plates. (**a**,**d**) Control groups; (**b**,**c**,**e**) Endophyte-phytopathogen antagonism tests.

3.2.3. Effect of Endophytes on the Growth of *Botrytis cinerea* (CGMCC: 3.3790)

The cultures of *Botrytis cinerea* were fast-growing, pale brown at the center, and white at the margins, and reached a diameter of around 60 mm in PDA after 10 days, without sporulating. *Daldinia* sp. KUNCC22-10744 strain was fast-growing and overlapped *Botrytis cinerea* (Figure 5b) with an inhibition rate of 55.56 ± 1.73% (Table 2). *Chaetomium* sp. KUNCC22-10749 strain inhibited the growth of *B. cinerea* (Figure 5c) with an inhibition rate of 52.02 ± 2.92% (Table 2) producing abundant aerial mycelium. *Alternaria* sp. KUNCC22-10760 strain inhibited the growth of *B. cinerea* (Figure 5d) with an inhibition rate of 67.15 ± 5.88% (Table 2) and the pathogen grew slowly, and mycelium was sparse.

3.2.4. Effect of Endophytes on the Growth of *Penicillium digitatum* (CGMCC: 3.15410)

The cultures of *Penicillium digitatum* were observed to be greenish, white at the margin, and a single colony in PDA reached 35–40 mm in diameter with sporulation after 10 days. *Daldinia* sp. KUNCC22-10744 strain was a fast-growing and overlapped pathogen (Figure 5f) with an inhibition rate of 51.16 ± 5.59% (Table 2). *Rosellinia* sp. KUNCC22-10751 strain was fast-growing while pathogens grew slowly (Figure 5g) at an inhibition rate of 60.83 ± 2.88% (Table 2). Figures 6–9 show the Inhibition rates of different fungal endophytes to GCMCC: 1.7276, GCMCC: 1.3333, GCMCC: 3.3790, and GCMCC: 3.15410.

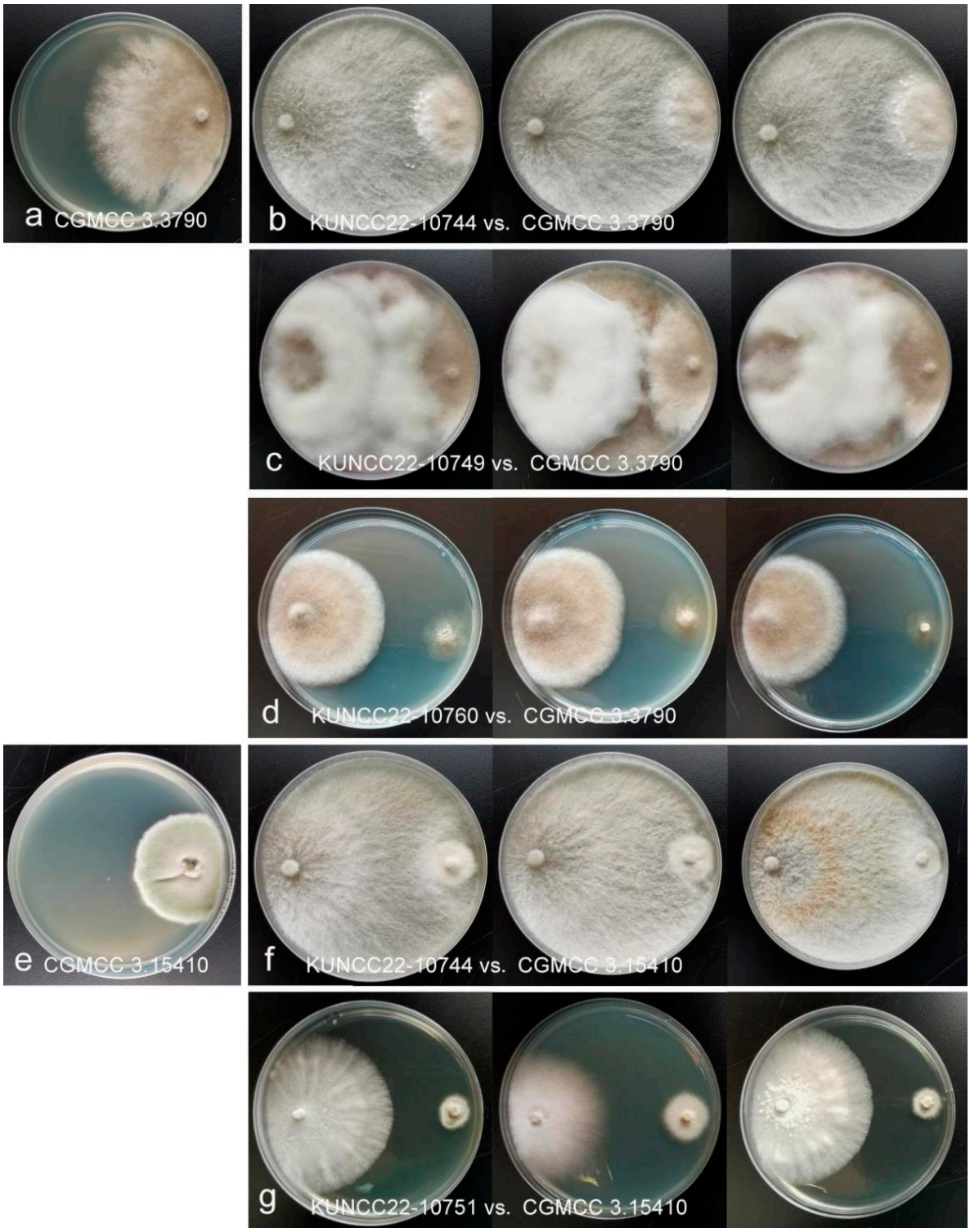

Figure 5. The two fungal pathogens co-cultivated with the mango fungal endophytes on PDA plates. (**a**,**e**) Control groups; (**b**–**d**,**f**,**g**) Endophyte-phytopathogen antagonism tests.

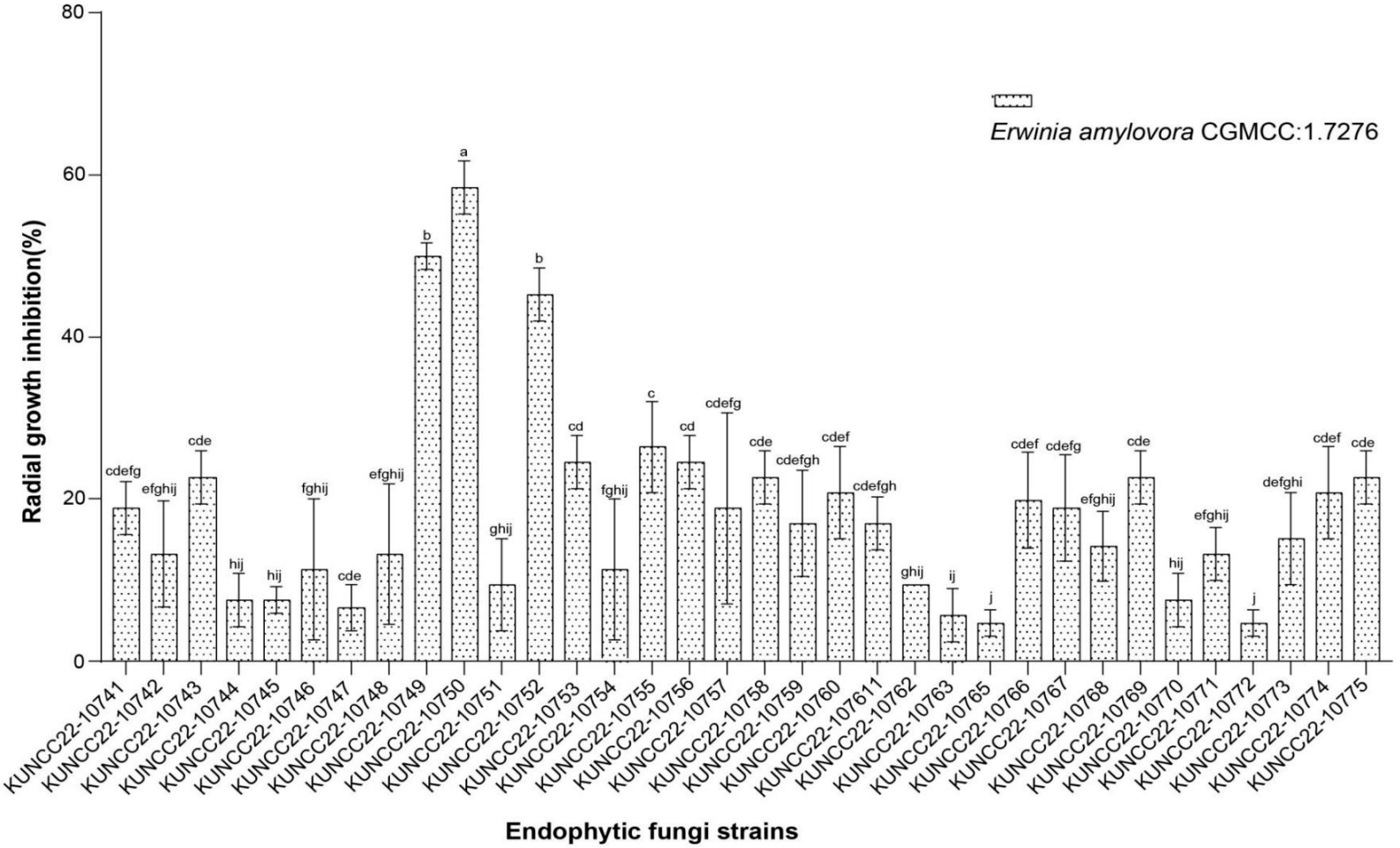

Figure 6. Inhibition rates of different fungal endophytes to bacterial pathogen *Erwinia amylovora* (GCMCC: 1.7276). The bars denoted by the same letter are not significantly different from each other.

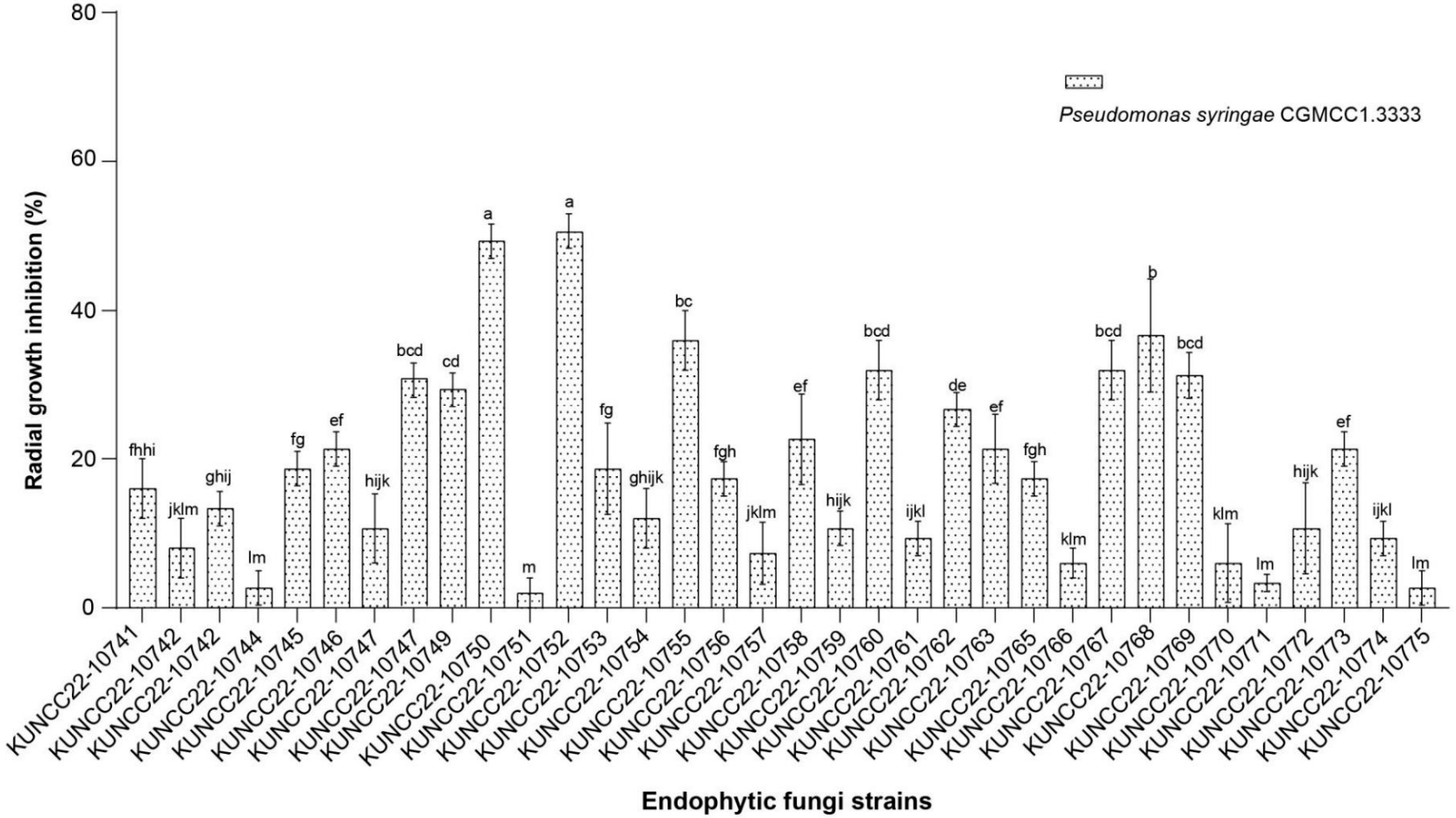

Figure 7. Inhibition rates of different fungal endophytes to bacterial pathogen *Pseudomonas syringae* (GCMCC1.3333). The bars denoted by the same letter are not significantly different from each other.

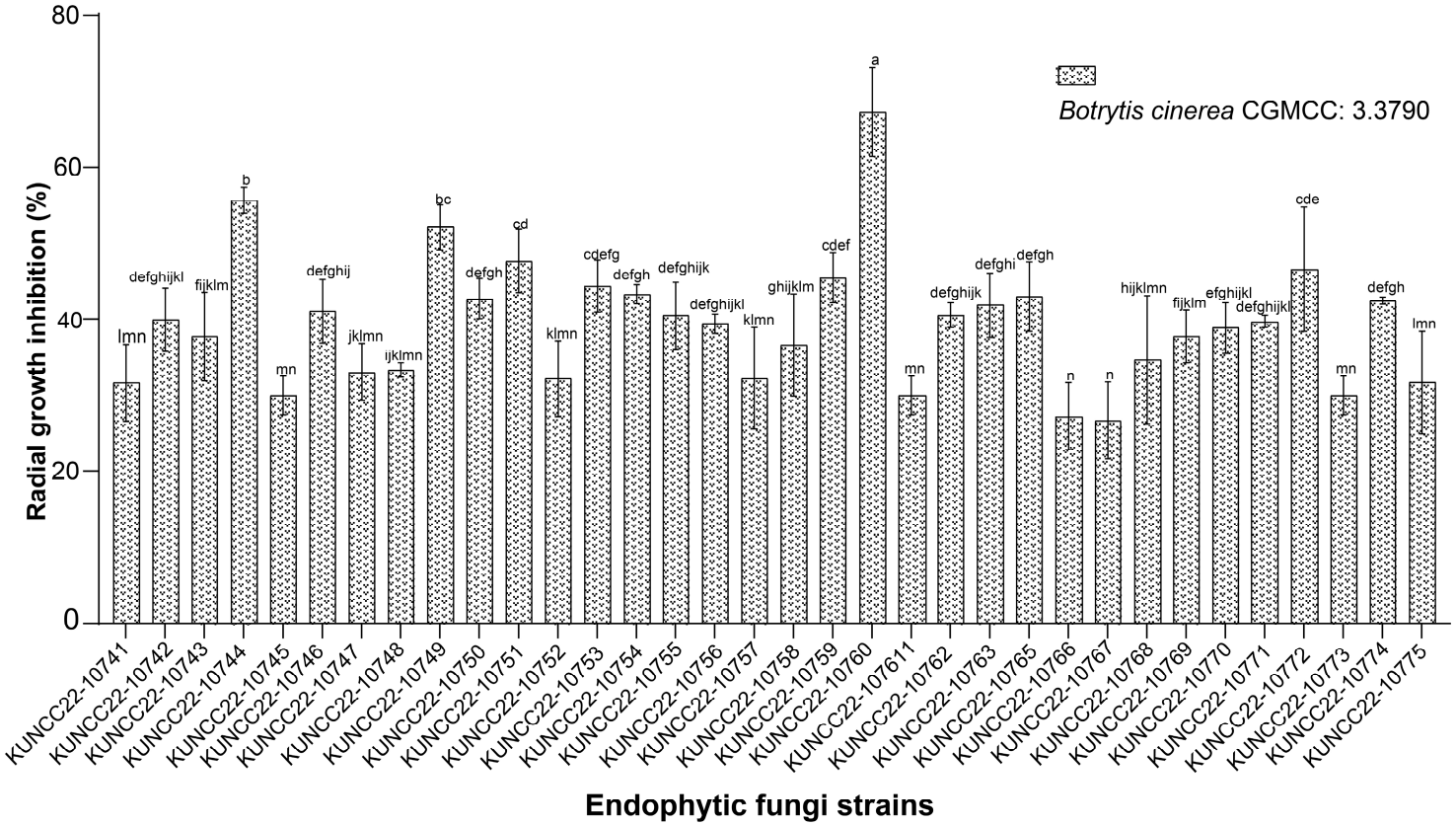

Figure 8. Inhibition rates of different fungal endophytes to fungal pathogen *Botrytis cinerea* (GCMCC: 3.3790). The bars denoted by the same letter are not significantly different from each other.

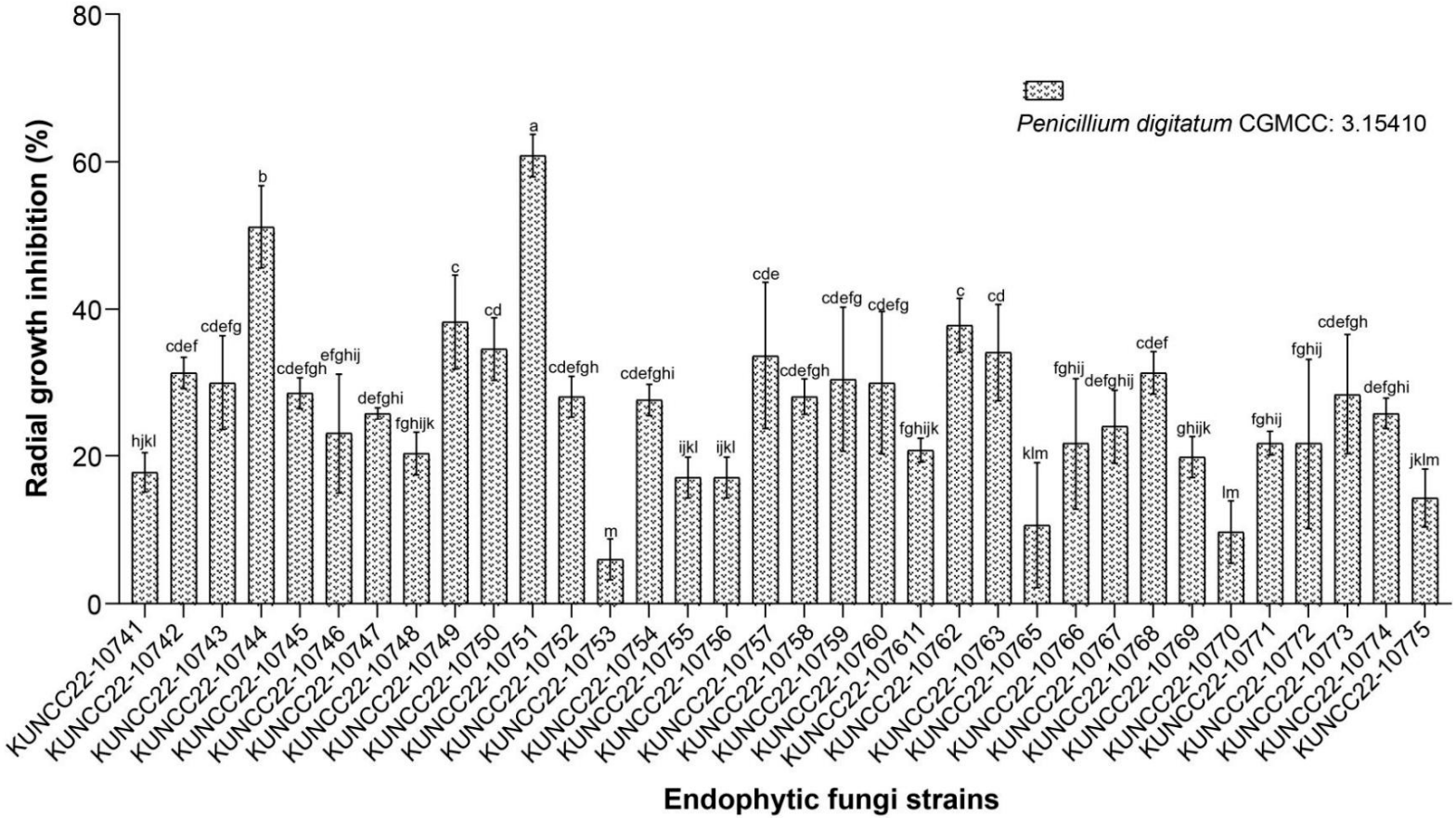

Figure 9. Inhibition rates of different fungal endophytes to fungal pathogen *Penicillium digitatum* (GCMCC: 3.15410). The bars denoted by the same letter are not significantly different from each other.

4. Discussion

The internal transcribed spacer (ITS) sequence has been generally recognized as a fungal barcode because it is the most sequenced region of fungi and is often used for identification, phylogenetics, and systematics [56,57]. Furthermore, the 5.8S-ITS region has previously been applied to the identification of endophytic fungal genera [58–61]. In this study, 34 isolates were identified to the generic level based on combined ITS. The nine different strains of *Chaetomium* were shown to be the dominant group, accounting for 26% of total strains (Figure 2). *Chaetomium* sp. strains viz. KUNCC22-10749, KUNCC22-10750, and KUNCC22-10752 showed antagonism against bacterial pathogens in vitro, while *Chaetomium* sp. KUNCC22-10749 is the only strain in this group that showed antagonism against the fungal pathogen *Botrytis cinerea* in vitro. *Chaetomium* is the largest genus in the family *Chaetomiaceae* and encompasses more than 350 species. Recently, this has become an important research topic due to its high diversity and prominent potential capability in biocontrol. The mechanisms of biocontrol of the endophytic *Chaetomium* spp. mainly include antibiosis, competition for nutrients and space, mycoparasitism, and induction of defense response in plants [62,63]. Species of *Chaetomium* have huge potential to control plant and soil fungal pathogens [64–66]. For example, *Chaetomium globosum* has been proven as a potential antagonist of *Cochliobolus sativus, Venturia inaequalis,* and *Pythium ultimum* [67–69]. *Chaetomium cupreum* and *C. globosum* have been reported to control corn leaf spot disease caused by *Curvularia lunata*, tomato wilt disease caused by *Fusarium oxysporum*, sheath blight disease of rice caused by *Rhizoctonia solani*, and rice blast disease caused by *Pyricularia oryzae* [70].

In this study, one of two *Daldinia* sp. strains KUNCC22-10744 strain exhibited significant antagonistic properties against the fungal pathogens *Botrytis cinerea* and *Penicillium digitatum* by competing for nutrition and space. *Daldinia* belongs to the family *Hypoxylaceae*, and currently, 58 species are accepted in this genus [66]. Specific secondary metabolites are constantly found in *Daldinia* sp., and they often have significant biological activities [71,72]. Liarzi et al. [73] isolated endophytic *Daldinia concentrica* from an olive tree, and its volatile organic compounds (VOCs) demonstrated antimicrobial activity against various fungi and oomycetes.

In this study, two *Alternaria* strains were isolated, and the *Alternaria* sp. (KUNCC22-10760 strain) was found to have prominent antagonistic properties against the fungal pathogen *Botrytis cinerea*. *Alternaria* as a pathogenic fungal group often causes black spot decay on hosts (including mango) [74,75]. However, Soltani and Hosseyni Moghaddam [76] isolated endophytic *Alternaria* species from healthy *Cupressaceae* trees, and a further study found their extracted metabolites exhibited significant growth inhibitory activities against pathogenic bacteria and fungi. These endophytic *Alternaria* are abundant in biologically active compounds that are possible to apply in medical and agricultural fields [77].

In this study, *Rosellinia* sp. KUNCC22-10751 strain exhibited prominent antagonistic abilities against the fungal pathogen *Botrytis cinerea*. Species of *Rosellinia* such as *Rosellinia bunodes*, *R. necatrix*, and *R. pepo* often cause root rots on many cash crops and trees [78]. Despite the fact that the endophytic species of *Rosellinia* have been shown to have potential as biocontrol agents, this is rarely reported [79]. Nevertheless, a large number of metabolites from endophytic *Rosellinia* species have been investigated recently [78,80,81].

5. Conclusions

Our study contributes to the knowledge of mango-associated endophytic fungi with the potential as biocontrol agents. We isolated 34 different fungal endophytes from healthy and fresh mango leaves, and the genus *Chaetomium* was reported as the dominant group. In addition, three strains of *Chaetomium* sp. showed great in vitro inhibition against two bacterial pathogens viz. *Erwinia amylovora* and *Pseudomonas syringae*, while the strains of *Alternaria* sp., *Chaetomium* sp., *Daldinia* sp., and *Rosellinia* sp. showed great to moderate in vitro antagonistic properties against fungi pathogens viz. *Botrytis cinerea* and *Penicillium digitatum*. Therefore, future studies should especially focus on how the studies trans-

late into in vivo action, such as inoculating those effective fungal endophytes on plant pots (Mango seedlings or *Arabidopsis thaliana*) that were infected by selected fungal or bacterial pathogens.

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