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Antifungal activity of endophytic *Streptomyces* strains from *Dendrobium* orchids and the secondary metabolites of strain DR7-3 with its genome analysis

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ABSTRACT

Chemical fungicides are widely used in the agriculture sector and cause severe environmental problems. Biological control, a method using antagonistic organisms, has been considered one of the most promising strategies to tackle this issue. Actinomycetes, particularly *Streptomyces* strains, produced diverse classes of bioactive secondary metabolites. In this study, four *Streptomyces* strains, DR5-1, DR7-3, DR8-5, and DR8-8, isolated from three *Dendrobium* species (Orchidaceae), exhibited significant antifungal activity against five phytopathogenic fungi, particularly with high potency against *Curvularia oryzae*. The bacterial identification was performed based on phenotypic and chemotaxonomic characteristics, including the 16S rRNA gene sequence. Strain DR7-3 from the roots of *Dendrobium findlayanum* exhibited high antifungal activity, and its culture filtrate caused damage to the cell structure of *C. oryzae* SA04. It was identified as *Streptomyces solisilvae* based on the average nucleotide identity, ANIb (98.49%), and DNA–DNA hybridization value (88.40%). The EtOAc extract from strain DR7-3 was analyzed by the gas chromatography-mass spectrometry method. Among the 15 identified compounds, eicosane, phenol-2,4-*bis*(1,1-dimethylethyl), hexadecane, and hexadecanoic acid-methyl ester showed significant antifungal activity. The draft genome sequence analysis of strain DR7-3 revealed 72 putative biosynthetic gene clusters of secondary metabolites. The genome alignment indicated that 13 gene clusters are involved in the biosynthesis of these antifungal metabolites. These results suggested that strain DR7-3 could be a promising candidate for developing new and safe microbial biological control agents for application in agricultural fields.

INTRODUCTION

In agricultural farming, plant diseases are one of the major causes of crop yield losses. During the past decades, chemical pesticides have been widely used to control and prevent infections. However, due to the growing resistance of phytopathogens, several chemical pesticides have markedly become less effective, and higher doses are needed to obtain the same results (Tirado *et al*., 2008). Moreover, many of these pesticide residues may pose potential risks to the health of farmers and consumers and serious environmental hazards. In Thailand, chemical fungicides are among the top three imported pesticides, with amounts of 12,000–15,000 tons per year (Office of Agricultural Economics, 2021). These chemicals, including dinocap, maneb, ferbam, benomyl, carbendazim, captafol, and tridemorph, are used to prevent and inhibit the growth of fungi, both before and after harvesting. The phytopathogenic resistance to these chemical fungicides has been continuously reported (Kongtragoul *et al*., 2021), and this problem has resulted in poor disease control. In addition, most of these fungicides are known to be hazardous to people, and their long-term usage may harm both the economy and the environment. There is an urgent need to promote organic farming and search for

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new biological agents to control plant diseases and replace these chemicals.

Actinomycetes are a promising source of agricultural biocontrol agents since they produce a vast array of secondary metabolites that can inhibit the growth of various bacterial and fungal phytopathogens without toxicity to humans and harm to the environment (Chitraselvi, 2018). Numerous reports revealed that endophytic actinomycetes could promote the growth of host plants and reduce disease symptoms in various environmental stresses through the activities of their secondary metabolites. For example, 3-methyl-3,5-amino-4-vinyl-2-pyrone, a broad-spectrum antifungal compound from *Streptomyces* sp. N2, was effective against anthracnose of grapefruits, a disease caused by the fungus *Colletotrichum gloeosporioides* (Xu *et al*., 2015). 2,5-*Bis*(hydroxymethyl)furan monoacetate, a furan derivative obtained from *Streptomyces* sp. CEN26, an endophytic actinomycete found in the root nodes of *Centella asiatica* (L.) Urban, inhibited and deformed the conidial germination of *Alternaria brassicicola*, a fungal pathogen of cabbage (Phuakjaiphaeo *et al*., 2016). Natamycin, a polyene macrolide isolated from the fermentation broth of *Streptomyces lydicus* AZ-55, inhibited the growth of the filamentous fungi *Fusarium oxysporum*, *Alternaria alternata*, and *Rhizoctonia solani* (Atta *et al*., 2015). Blasticidin-S, an antibiotic from *Streptomyces griseochromogenes*, has been used commercially in Japan to reduce rice blasts (Tapadar and Jha, 2013). In a recent study, the crude extracts from the culture filtrate of *Streptomyces* sp. SCA2-4^T destroyed the cell structure and inhibited mycelial growth and spore germination of the banana wilt fungus *F. oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) (Qi *et al*., 2021). However, it should be noted that if these biological control agents are to be employed to treat several kinds of phytopathogenic infections, new and potent secondary metabolites with a broad-spectrum activity must be obtained. In this regard, whole-genome sequencing analysis has been a useful tool for identifying important biocontrol agents (Zerikly and Challis, 2009).

Endophytes are ubiquitous and remain in specific association with the host plants, for example, mutualism or antagonism but not parasitism (Nair and Padmavathy, 2014). Endophytic actinobacteria have been reported from various plants, including dominant *Streptomyces*, along with other genera *Micromonospora, Microbispora*, *Nocardia*, *Nocardioides*, and *Streptosporangium* (Shimizu, 2011). It has been demonstrated that plant-associated microorganisms create compounds of therapeutic potential (Wu *et al*., 2021). However, there have been few investigations on the endophytic microorganisms in orchids. For example, two new cytotoxic and antifungal chemicals isolated from the endophytic fungus *Pestalotiopsis* sp. DO14 of *Dendrobium officinale* are excellent resources for preventing or treating pathogens (Wu *et al*., 2015). Fungal endophytes from seven *Dendrobium* species demonstrated a powerful and diverse antibacterial range (Cui *et al*., 2012) and from *D. devonianum* and *D. thyrsiflorum* exhibited both antibacterial and antifungal activity against pathogenic organisms (Xing *et al*., 2011). This study deals with the identification and evaluation of the antifungal activity of endophytic *Streptomyces* from three *Dendrobium* orchids. Four isolates are recognized based on their phenotypic, chemotaxonomic, and genotypic characteristics. The identification of secondary metabolites from the most promising strain and its genome analysis are discussed.

MATERIALS AND METHODS

Isolation, cultivation, and maintenance of actinomycetes

Actinomycetes were isolated from the roots of three *Dendrobium* orchids: *D. kentrophyllum, D. findlayanum,* and *D. chrysanthum.* The roots were washed with running tap water and dried at room temperature and prepared according to our previously reported protocols (Tedsree *et al*., 2021). The suspension was serially diluted 10 times. Each diluted suspension was spread on starch casein gellan gum (Küster and Williams, 1964) supplemented with nalidixic acid (25 µg/ml) and cycloheximide (50 µg/ml) (Kuncharoen *et al*., 2019). Bacterial colonies were picked up and purified after being incubated at 30°C for three weeks. The purified strains were preserved on ISP 2 slants and freeze-dried for long-term storage.

Phenotypic characteristics

Cell morphologies of the isolates were investigated by light microscopy (CX41, Olympus) and scanning electron microscopy (JSM-IT500HR, JEOL) after being cultivated on ISP 2 agar plates at 30°C for 14 days. The cultural characteristics were observed on ISP 2 agar as described previously (Shirling and Gottlieb, 1966). The NBS/IBCC color system was used to determine the colors of aerial mycelia, substrate mycelia, and diffusible pigment (Kelly, 1964). Physiological characteristics, including the growth at different temperatures (20°C–45°C), NaCl concentrations (0%–10%, w/v), and pH range of 4–12 (at intervals of 1 pH unit), were evaluated in ISP 2 broth at 30°C for 14 days. Carbon utilization on ISP 9 supplemented with 1% (w/v) carbon sources, starch hydrolysis, nitrate reduction, milk coagulation, peptonization, and gelatin liquefaction was examined as described by Arai (1975). The isomer of diaminopimelic acid in cell wall peptidoglycan was determined as described by Staneck and Roberts (1974).

16S rRNA gene sequence analysis

Genomic DNAs of the isolates were generated using the technique earlier described (Kudo *et al*., 1998). The 16S rRNA gene sequence was amplified according to a well-established method (Suriyachadkun *et al*., 2009) and then sequenced on a DNA sequencer (Macrogen) using universal primers, 27F forward (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R reverse (5′-TACGGYTACCTTGTTACGACTT-3′). The sequence similarity values between the isolates and their related neighbors were calculated using the EzBioCloud service (Yoon *et al*., 2017).

Evaluation of antifungal activity

The isolates were cultivated in the broth of medium no. 57 (0.5% peptone, 2% glucose, 0.5% meat extract, 0.3% dry yeast, 0.5% NaCl, and 0.3% $CaCO_3$) at 180 rpm, 30°C. After 14 days, the metabolites were extracted with 95% ethanol, and the cell suspension was centrifuged. The supernatant was used for testing the antifungal activity. The medium without the culture was used as a negative control. Antifungal activity was determined using the agar disc diffusion method (Mearns-Spragg *et al*., 1998). The

tested filamentous fungi, *F. oxysporum* SA01, *Fusarium solani* SA02, *Alternaria alternata* SA01, *C. gloeosporioides* SA03, and *Curvularia oryzae* SA04, were cultivated on potato dextrose agar (PDA) plates. The paper disc was added with the supernatant amount of 50 µl and dried at room temperature. The actinomycete discs and phytopathogenic fungal disc were placed at a position 2.0 cm from the edge and center of the plate, respectively. The plates were incubated at 30°C until the fungal mycelium in the control plate grew to the edge of the plate. The inhibition zone was measured as the distance between the actinomycete discs and fungal mycelium edge. The percentage of inhibition is $[(C - T)/C] \times 100$, where C is the fungal radius in the control plate and T is the fungal radius in the treatment plate. All experiments were carried out in triplicate.

Fermentation and extraction

The seed strain was cultivated in ISP 2 broth on a shaker (180 rpm), at 30°C, for 3 days and then was transferred into the broth of medium no. 57 on a shaker (180 rpm), at 30°C for 14 days. The culture broth of DR7-3 was extracted with ethyl acetate three times. The ethyl acetate layer was evaporated to dryness under a vacuum. The crude extract was stored in a dark and cold place for further tests.

Antifungal activity on the mycelial growth

The crude extract was determined for antifungal activity against the phytopathogenic fungus *C. oryzae* SA04. The percentage of mycelial inhibition was obtained using the poisoned food technique. The fungi were cultured on a PDA medium. The crude extract was prepared as a solution in dimethyl sulfoxide (DMSO) in different concentrations. The warm PDA medium of 45°C–50°C was added with different concentrations of the crude extract and then poured into sterilized Petri dishes. The control plate was a PDA medium with DMSO. The phytopathogenic fungal disc was placed at the center of the plate, for both control and treatment plates. The plates were incubated at 30°C until the fungal mycelia in the control plate reached the edge of the plate. The percentage of mycelial inhibition was calculated from the formula $[(C - T)/C] \times 100$, where C is the colony diameter in the control plate and T is the colony diameter in the treatment plate. The results were compared with the antifungal activity of benomyl, a commercial chemical fungicide. To calculate the IC_{50} value, an online tool "Quest Graph™ IC_{50} Calculator" (by AAT Bioquest) was used (https://www.aatbio.com/tools/ic50-calculator).

The antifungal effect of crude extract DR7-3 on *C. oryzae* SA04 mycelia was determined using scanning electron microscopy (SEM, JSM-IT500HR, JOEL). The fungal mycelia samples (direct contact with crude of strain DR7-3 at IC_{50}) and control (without the crude) were cut and examined by SEM.

Chemical profile of the crude extract

The chemical profile of the crude extract of the strain DR7-3 was investigated using gas chromatography (GC-MS). The analysis was carried out with GC Agilent 6890/MS Hewlett 5973. The injection port temperature was maintained at 250°C, and the column oven temperature program was set at 40°C for 2 minutes and then increased to 250°C (5°C/minute), ending with a 20 minutes isothermal process at 250°C. The carrier gas was helium (1 ml/minute), HP-5MS (30 m × 0.32 mm × 0.25 µm) was the stationary phase, and an injection volume of 1 µl was used. The chemical components were identified by comparison of their mass fragmentation patterns to those of the standard reference data of NIST libraries.

Genomic analyses of DR7-3

Whole-genome sequence analysis of the selected strain was performed with the Illumina MiSeq platform (Illumina, Inc., San Diego, CA) using 2 × 250 bp paired-end reads. The assembling of the reads to contigs was managed using SPAdes 3.12 (Bankevich *et al*., 2012). The draft genome of strain DR7-3 was determined using the antiSMASH server (Blin *et al*., 2019) to detect putative biosynthetic gene clusters (BGCs). All genomes were annotated on Prokka software 1.13 (Seemann, 2014) in line with the NCBI Prokaryotic Genome Annotation Pipeline. A phylogenomic tree of strain DR7-3 and its closest type strains was constructed using the TYGS web server (https://tygs.dsmz.de/) (Meier-Kolthoff and Göker, 2019). Average nucleotide identity (ANI), ANI-BLAST (ANIb), and ANI-MUMmer (ANIm) values between strain DR7-3 and closely related type strains were calculated pairwise using the JSpeciesWS web service (Richter *et al*., 2016). The digital DNA–DNA hybridization (dDDH) was evaluated using the Genome-to-Genome Distance Calculator (GGDC 2.1) with the BLAST+ method (Meier-Kolthoff *et al*., 2013), and the results were dependent on recommended formula 2 (identities/HSP length), which is proposed for use with incomplete whole-genome sequences.

Statistical analysis

Data were statistically analyzed by analysis of variance using the SPSS software package (SPSS 28 for Windows). The grouping was performed by Duncan's multiple range tests at $p = 0.05$ on each of the significant variables measured. The data were expressed as mean values of triplicates ± standard deviation.

RESULTS

Isolation and identification of isolates

Four endophytic actinomycetes were recovered from the roots of the Thai orchid. Strains DR5-1 and DR7-3 were isolated from *D. kentrophyllum* and *D. findlayanum*, respectively, whereas strains DR8-5 and DR8-8 were obtained from *D. chrysanthum*. Results from the 16S rRNA gene sequence and phenotypic characteristic data indicated that all the isolates were *Streptomyces* spp. The pairwise alignment of the 16S rRNA gene sequence among all had 99.15%-99.93% similarity. Strains DR5-1, DR7-3, DR8-5, and DR8-8 were closely related to *S. pravulus* NBRC 13193^T (99.40%), *S. solisilvae* HNM0141^T (99.15%), *S. daghestanicus* NRRL B-5418^T (99.93%), and *S. malaysiense* MUSC 136^T (99.85%), based on 16S rRNA gene sequence similarity. The 16S rRNA gene sequences of these strains have been deposited in the NCBI database, and their accession numbers are listed in Table 1. The strains produced spiral spore chains with pale blue to gray color on ISP 2 agar after seven-day incubation. The scanning electron micrograph of strain DR7-3 revealed spiral spore chains on aerial hyphae (Fig. 1). All strains contained LL-diaminopimelic acid (Williams and Cross, 1971). The optimum temperature of all

Table 1. Phenotypic characteristics of isolates.

Characteristic	Isolate no.			
	DR5-1	**DR7-3**	**DR8-5**	**DR8-8**
Accession no.	LC685847	LC685849	LC685851	LC685853
Upper color	Pale blue	Bluish gray	Greenish white	Bluish gray
Reverse color	Light yellow	Light yellow	Light yellow	Light yellow
pH range	5–10	5–10	5–7	5–10
NaCl (%)	8	6	10	6
Starch hydrolysis	+	+	+	+
Coagulation	+	–	–	–
Nitrate reduction	–	+	–	+
Gelatin liquefaction	+	+	+	+
Utilization of				
D-Glucose	+	+	+	+
Sucrose	+	+	w	+
Lactose	+	+	+	+
D-Fructose	+	+	+	+
Maltose	+	+	+	+
D-Mannitol	+	+	+	+
D-Xylose	+	+	+	+
D-Sorbitol	–	W	w	w

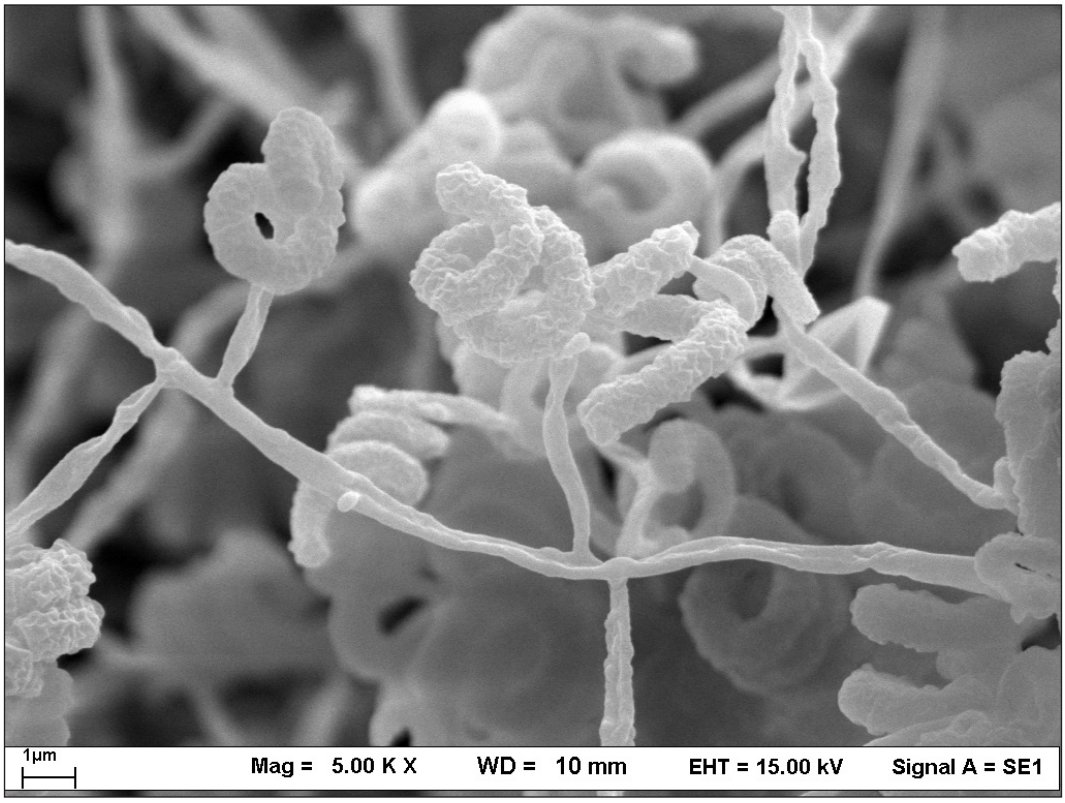

Figure 1. Scanning electron micrograph of strain DR7-3 grown on ISP 2 agar at 30°C for 14 days.

strains was 25°C–30°C, and the pH range was 5–10. All strains utilized various sugars for growth and grew in a range of 5%–10% NaCl concentrations. The cultural, physiological, and biochemical characteristics of the isolates are shown in Table 1.

Orchids are known to be a rich source of endophytic microorganisms. Studies on the tissues of several terrestrial orchids indicated that the number and type of endophytes follow the seasonal rhythm of the year (Chutima *et al*., 2011). In this study of *Dendrobium* orchids, *Streptomyces* was found to be a dominant genus, similar to a previous report (Tsavkelova *et al*., 2007). In addition, *Streptomyces* sp. viji10 was reported from the velamen roots of the *Vanda spathulata* orchid (Senthilmurugan *et al*., 2013), while *Actinomycetospora endophytica* was identified as a novel species from the wild orchid *Podochilus microphyllus* (Sakdapetsiri *et al*., 2018).

Evaluation of antifungal activity

The antagonistic activity of the endophytic *Streptomyces* in this study was investigated *in vitro* against a wide range of phytopathogens using the dual culture technique. The inhibitory activities of strains DR5-1, DR7-3, DR8-5, and DR8-8 against five phytopathogenic fungi are shown in Table 2.

Among the four *Streptomyces* isolates in this study, DR7-3 presented the most interesting result, showing the highest antifungal potential, with 35.05 ± 0.95% inhibition against *C. oryzae* SA04, and thus was selected for further detailed studies (see below). Strain DR5-1 also exhibited remarkable antifungal activity, with the highest effects observed in three fungi, including against *A. alternata* SA01, *F. solani* SA02, and *C. gloeosporioides* SA03, whereas DR8-5 was the strongest isolate when tested against *F. oxysporum* SA01.

Endophytic actinomycetes from several sources have been studied earlier for their inhibitory activity against phytopathogenic fungi. For example, *Streptomyces* sp. CMUAc130 isolated from *Zingiber officinale* could inhibit *Colletotrichum musae* and *F. oxysporum* (Taechowisan and Lumyong, 2003), *Streptomyces* sp. S12-10 from rice showed high percentages of inhibition against *Fusarium moniliforme, Helminthosporium oryzae*, and *R. solani*, whereas *Streptomyces* strain CEN26 isolated from *C. asiatica* (L.) displayed significant antifungal activity against *A. brassicicola* (Phuakjaiphaeo and Kunasakdakul, 2015). In a more recent study, wetland-derived *Streptomyces* sp. ActiF450 exhibited a broad-spectrum antifungal activity against *Aspergillus niger* MA2, *F. oxysporum* F15, *Penicillium chrysogenum* ICF59, and *Scopulariopsis candida* ICF53 (Benhadj *et al*., 2020). The inhibitory potential of soilborne *Streptomyces hygroscopicus* against the fungus *C. gloeosporioides* was described earlier (Prapagdee *et al*., 2008). This study constitutes the first report of the antifungal activity of endophytic actinomycetes isolated from *Dendrobium* orchids.

Antifungal activity on the mycelial growth

The extract from the strain DR7-3 inhibited the mycelial growth of *C. oryzae* SA04 in a dose-dependent manner (Table 3). The colony growth was suppressed (100% inhibition) when the concentration of the extract reached 3,000 ppm. The IC_{50} value of the extract against *C. oryzae* SA04 was 25.75 ppm, significantly lower than that of the chemical fungicide benomyl (178.5 ppm). Benomyl has been widely used against a variety of phytopathogenic fungi (Tobih *et al*., 2015), and its fungicide action was related to its capacity to be absorbed by phytopathogen cells (Summerbell, 1993).

Scanning micrograph analyses revealed that the *C. oryzae* SA04 mycelia taken from the colony's edge differed from the control. The control has a typical structural feature, such as a smooth outer surface on the cylindrically formed mycelium (Fig. 2A), whereas mycelia treated with DR7-3 extract were severely deformed, with uneven shrinkages, roughness, loss of smoothness, and a swollen mycelium surface (Fig. 2B). The unusual morphology of fungal hyphae can be taken as evidence of the antifungal activity of the test sample (Hashem *et al*., 2016). In a previous report, the fungicidal activity of a butanol extract of *Streptomyces blastmyceticus* 12-6 on *Colletotrichum acutatum* and *F. oxysporum* was studied using the SEM technique, which showed the abnormal morphology of hyphae, such as swelling and a reduction in cytoplasmic content, with apparent separation of the cytoplasm from the cell wall (Kim *et al*., 2019). These phenomena were also observed in *C. oryzae* PSUNK1012 when treated with the culture filtrate of *S. angustmyceticus* (Pithakkit *et al*., 2015). Similar observations were also reported for *F. oxysporum* race 4 upon adding the extract of *Streptomyces* sp. CB-75 (Chen *et al*., 2018).

Table 2. Antifungal activity of isolates.

Isolate no.	% inhibition				
	***F. oxysporum* SA01**	***F. solani* SA02**	***A. alternata* SA01**	***C. gloeosporioides* SA03**	***C. oryzae* SA04**
DR5-1	21.85 ± 1.67^{h}	29.17 ± 0.96^{cd}	24.29 ± 0.86^{g}	27.33 ± 0.72^{cdef}	29.55 ± 0.83^{cd}
DR7-3	13.70 ± 1.12^{k}	25.37 ± 1.40^{fg}	19.24 ± 1.00^{j}	26.57 ± 1.31^{efg}	35.05 ± 0.95^{a}
DR8-5	24.81 ± 1.97^{g}	28.70 ± 2.89^{cde}	11.90 ± 0.44^{k}	21.52 ± 0.72^{hi}	29.64 ± 1.22^{c}
DR8-8	19.54 ± 1.53^{ij}	27.22 ± 1.21^{def}	17.33 ± 1.15^{j}	25.24 ± 0.87^{fg}	32.52 ± 0.95^{b}

The different letters mean significant difference ($p < 0.05$), mean ± SD.

Table 3. Antifungal activity of ethyl acetate extract against *C. oryzae* SA04 of strain DR7-3.

Treatments	% Inhibition								
	0 ppm	**100 ppm**	**200 ppm**	**500 ppm**	**800 ppm**	**1,000 ppm**	**2,000 ppm**	**3,000 ppm**	**IC_{50}**
Crude DR7-3	0.00	69.40	75.40	81.80	82.60	83.20	95.00	100.00	25.75
Benomyl	0.00	37.60	43.40	44.00	50.00	60.60	71.00	80.00	178.5

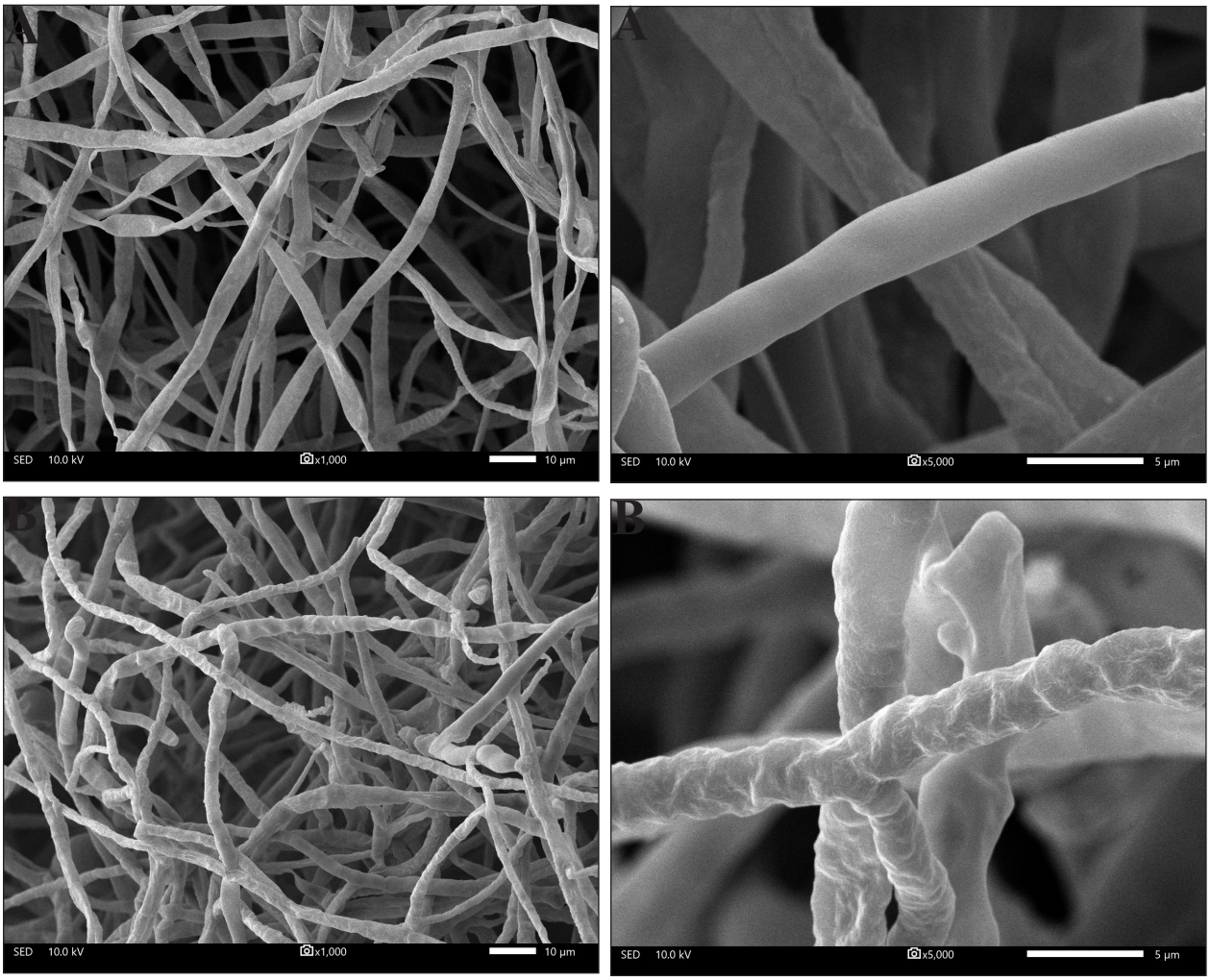

Figure 2. Scanning electron micrograph of mycelia of *C. oryzae* SA04 without treatment (A and magnified A); treatment with ethyl acetate extract from strain DR7-3 (B and magnified B).

Genomic sequencing analysis

Genome analysis of strain DR7-3 revealed the size of 11,331,527 bp distributed in 159 contigs with a G+C content of 71.12% and 9,582 protein-coding sequences (CDSs). The phylogenetic analysis based on whole-genome sequences (Fig. 3) indicated that strain DR7-3 was phylogenetically closed to *S. solisilvae* HNM0141^T. The ANIb and ANIm of the genomes DR7-3 and *S. solisilvae* HNM0141^T were 98.49% and 98.71%, respectively. The dDDH values were the highest, 88.40% with *S. solisilvae* HNM0141^T. For genome comparison, ANI and dDDH values are considered well correlated when the values were ≥95% (ANI) and ≥70% (dDDH), respectively (Fitch, 1971; Seemann, 2014). Since the dDDH (90.90%) and the ANI (98.59%–99.03%) values between strain DR7-3 and *S. solisilvae* HNM0141^T were higher than the species cut-off, DR7-3 was identified as *S. solisilvae.* The GenBank accession number for the draft genome sequence of strain DR7-3 is JAMQOH000000000.

Gene function annotation and secondary metabolism gene clusters

The draft genome of strain DR7-3 was determined using the antiSMASH server to detect putative BGCs. More than 70 gene clusters were observed on DR7-3 genomes related to various BGCs, mainly type I polyketide synthase (T1PKS), nonribosomal peptide synthetase (NRPS), terpene, and siderophore (Table 4). The secondary metabolite biosynthetic gene clusters (smBGCs) exhibited 100% similarity in genetic relatedness to the known clusters producing geosmin, desferrioxamine B, ectoine, coelichelin, pristinol, and echoside. Interestingly, desferrioxamine B, echoside A, and echoside B have a potential as SARS-CoV-2 inhibitors (Bellotti and Remelli, 2021; Melinda *et al*., 2021). Thus, strain DR7-3 might be one of the sources of natural anti-COVID-19 compounds. Strain DR7-3 is predicted to produce anticancer agents such as geldanamycin (Fukuyo *et al*., 2010), salinomycin (Antoszczak and Huczyński, 2015), and hygrocin A/ hygrocin B (Yin *et al*., 2017). The predicted secondary metabolites meilingmycin and herboxidiene can be used as insecticides (Sun *et al*., 2003) and herbicides (Pokhrel *et al*., 2015; Wideman, 1992), respectively, in agricultural farming.

Comparing the BGCs of DR7-3 to those of the close strain *S. solisilvae* HNM0141^T revealed similar main gene clusters. However, the numbers of some gene clusters, such

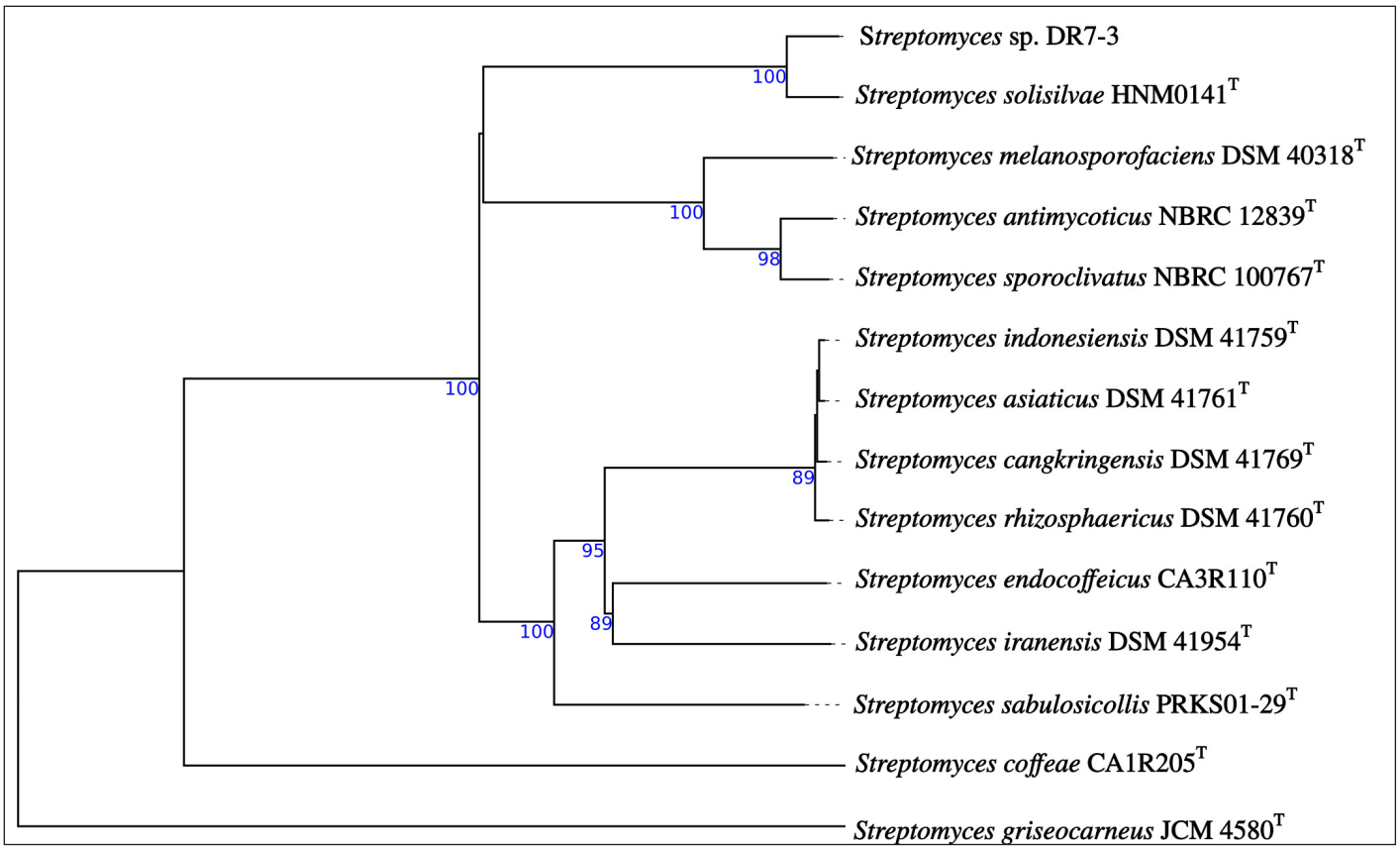

Figure 3. Phylogenomic tree of strain DR7-3 and related *Streptomyces* species obtained from TYGS. The numbers above branches are GBDP pseudo-bootstrap support values from 100 replications.

as T1PKS and NRPS-like, were different. T1PKS and NRPS-like in DR7-3 predicted secondary metabolites not found in *S. solisilvae* HNM0141^T (Table 4). The strain DR7-3 exhibited genetic relatedness to five known smBGCs that are associated with antifungal activity, rustmicin, elaiophylin, coelichelin, cyphomycin, and rapamycin, and found in *S. solisilvae* HNM0141^T. However, eight smBGCs, heronamide, niphimycins, fluvirucin B2, primycin, sceliphrolactam, niphimycins, pentamycin, and mediomycin A, were present only in strain DR7-3. In addition, the genome of strain DR7-3 contained nine smBGCs that displayed no similarity to any known smBGCs in antiSMASH (Table 4). These results suggested that strain DR7-3 might be a source of novel secondary metabolites with antifungal activity.

Table 4. Comparison of BGCs composition of strain DR7-3 and *S. solisilvae* HNM0141^T.

Type	Strain	
	DR7-3	*S. solisilvae* HNM0141^T
NRPS-like	5	2
Terpene	5(1)	5(1)
NRPS	3(1)	4
T1PKS	25(2)	9(1)
Siderophore	1(2)	1(2)
Butyrolactone	1(1)	1(1)
Redox-cofactor	1	1
Hserlactone	1	1
NAPAA	1	1
RiPP-like	(1)	(1)
Ectoine	1	1
Ladderane	1	1
Indole	1	1
RRE-containing	1	1
Hybrid	15(1)	14(1)

NRPS: nonribosomal peptide synthetases; NRPS-like: NRPS-like fragment; T1PKS: type 1 polyketide synthetases; NAPAA: non-alpha poly-amino acids like e-polylysine; RiPP-like: other unspecified ribosomally synthesized and posttranslationally modified peptide products; RRE-containing: RRE-element containing. Values in brackets indicate the number of clusters nonsimilar to those in the database.

Identification of bioactive compounds of strain DR7-3 by GC-MS

The secondary metabolites of the strain DR7-3 extract were analyzed by GC-MS. A total of 15 chemical compounds were identified by alignment of the NIST library based on retention time, molecular mass, molecular formula, and their biological activity (Table 5). These compounds were identified as 1) 2,3-butanediol, 2) 4-hydroxy-4-methyl-2-pentanone, 3) phenyl ethanol, 4) phenyl propanoic acid, 5) 2-phenylethyl ester of acetic acid, 6) *N*-tetradecane, 7) 2,4-*bis*(1,1-dimethylethyl) phenol, 8) hexadecane, 9) 1-heptadecane, 10) *n*-octadecane, 11) 2-phenylethyl ester of phenyl-acetic acid, 12) methyl ester of hexadecanoic acid, 13) 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo, 14) eicosane, and 15) *bis*(2-ethylhexyl) *ester of hexanedioic acid* ester of hexanedioic acid.

Table 5. Chemical profile and bioactivity of ethyl acetate extract of strain DR7-3.

No.	RT	Compound name	MW	MF	Similarity (%)	Bioactivity
1	5.48	2,3-Butanediol	90.07	$C_4H_{10}O_2$	91	Antibacterial activity (Wu *et al*., 2019)
2	6.85	4-Hydroxy-4-methyl-2-pentanone	116.08	$C_6H_{12}O_2$	83	No activity reported
3	14.61	Phenyl ethanol	122.07	$C_8H_{10}O$	94	Antibacterial activity (Corre *et al*., 1990)
4	16.89	Phenyl propanoic acid	150.07	$C_9H_{10}O_2$	50	Antineuroinflammatory activities (Li *et al*., 2019)
5	19.17	2-Phenylethyl ester of acetic acid	164.08	$C_{10}H_{12}O2$	90	Antimicrobial activity (Tayade & Jadhao, 2012; Valsalam *et al*., 2019)
6	23.10	*N*-Tetradecane	198.24	$C_{14}H_{30}$	98	Antimicrobial activity, antituberculosis (Girija *et al*., 2014)
7	25.78	2,4-*bis*(1,1-Dimethylethyl) phenol	206.17	$C_{14}H_{22}O$	94	Anti-QS and antibiofilm activities (Padmavathi *et al*., 2014); antifungal activity (Rangel-Sánchez *et al*., 2013; Ren *et al*., 2019)
8	28.04	Hexadecane	226.27	$C_{16}H_{34}$	98	Antifungal, antibacterial, antioxidant (Yogeswari *et al*., 2012)
9	32.33	1-Heptadecane	238.27	$C_{17}H_{34}$	91	No activity reported
10	32.48	*n*-Octadecane	254.30	$C_{18}H_{38}$	98	No activity reported
11	34.82	2-Phenylethyl ester of phenyl-acetic acid	240.12	$C_{16}H_{16}O_2$	91	No activity reported
12	35.03	Methyl ester of hexadecanoic acid	270.26	$C_{17}H_{34}O_2$	99	Antifungal (Kawuri and Darmayasa, 2019); antibacterial, antioxidant, antitumor, immunostimulant, and lipoxygenase inhibitor (Rahbar *et al*., 2012)
13	35.07	1,4-Diaza-2,5-dioxo-3-isobutyl bicyclo	210.14	$C_{11}H_{18}N_2O_2$	64	Cytotoxic activity (Narendhran *et al*., 2014); antifungal (Hanif *et al*., 2017)
14	36.50	Eicosane	282.33	$C_{20}H_{42}$	99	Antifungal activity (Karanja *et al*., 2010; Nandhini *et al*., 2015)
15	43.37	*bis*(2-Ethylhexyl) ester of hexanedioic acid	370.31	$C_{22}H_{42}O_4$	91	Antimicrobial activity, antioxidant, antiproliferative (Kadhim *et al*., 2017; Paramanantham and Murugesan, 2014)

Among these compounds, eicosane, 2,4-*bis*(1,1-dimethylethyl) phenol, hexadecane, and methyl ester of hexadecanoic acid have been reported earlier to possess antifungal activity (Table 5).

Eicosane, a long-chain fatty acid, was detected in the crude extract of *Streptomyces* sp. KX852460 showed antifungal activity against *R. solani* AG-3 KX852461, the cause of leaf spot disease (Ahsan *et al*., 2017). This compound is also present in the flower of *Allium atroviolaceum*, contributing to the antimicrobial activity of the plant extract (Dehpour *et al*., 2012). 2,4-*Bis*(1,1-dimethylethyl)-phenol from the ethyl acetate extract of *Kutzneria* sp. TSII inhibited the pathogenic fungus *Pithomyces atro-olivaceous* (Devi *et al*., 2021). This compound was produced by *Pseudomonas fluorescens* TL-1 and showed antifungal activity against *Curvularia lunata* (Ren *et al*., 2019). The long-chain hydrocarbon hexadecane from *Jatropha curcas* leaf extracts was exhibited against groundnut late leaf spot disease caused by *Phaeoisariopsis personata* (Francis *et al*., 2021). Hexadecanoic acid-methyl ester, a long-chain fatty ester produced from *Streptomyces galbus* TP2 and *Streptomyces humidus*, has been identified as an antifungal constituent (Kawuri and Darmayasa, 2019). It should be noted that *bis*(2-ethylhexyl)-hexanedioic acid, the major compound in the DR7-3 extract, possessed antimicrobial, antioxidant, and antiproliferative activities (Kadhim *et al*., 2017; Paramanantham and Murugesan, 2014).

CONCLUSION

The endophytic actinomycetes DR5-1, DR7-3, DR8-5, and DR8-8 isolated from three *Dendrobium* orchids belong to the genus *Streptomyces*. They showed inhibitory activity against several phytopathogenic fungi. Strain DR7-3 was identified as *S. solisilvae* and exhibited a broad-spectrum antifungal activity against five fungi that are causal agents of plant diseases. The ethyl acetate extract from this strain showed a high level of inhibition against *C. oryzae* SA04 compared with a standard chemical fungicide. Moreover, it suppressed mycelial growth and damaged the cell structure of the fungi. Four chemical components with antifungal activity were identified from the extract using the gas chromatography-mass spectrometric (GC-MS) technique. The draft genome sequence analysis of strain DR7-3 indicated that 13 gene clusters are involved in the biosynthesis of these antifungal metabolites. In our investigation, *S. solisilvae* DR7-3 appears to be a promising source for developing new antifungal agents against phytopathogenic fungi.

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CONFLICTS ON INTEREST

The authors declare that there are no conflicts of interest.

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ETHICAL APPROVAL

This article does not contain any studies with human participants and/or animals performed by any authors. Formal consent was not required in this study.

AUTHORS' CONTRIBUTIONS

All authors have made significant contributions to the conceptualization and design, data acquisition, data analysis and interpretation, and revision of the manuscript.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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