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Identification of *Pilidium lythri* and screening of fungicides *in vitro*

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Abstract: In recent years, strawberry tan-brown leaf spot disease caused by *Pilidium lythri* is a serious new disease that has occurred in China. Between 2015 and 2017, a total of 90 symptomatic samples were collected from Changping District, Beijing City; Zhucheng County, Shandong Province; and Loudi City, Hunan Province in China, with 30 samples from each district. Twenty-six fungal isolates were identified as *P. lythri* based on morphology, molecular biology, and phylogenetic analyses. This is the first report of *P. lythri* caused strawberry tan-brown spot in Shandong and Hunan Provinces in China. At present, there are no registered fungicides in China for controlling tan-brown spot disease on strawberry caused by *P. lythri*. In this study, the susceptibility of 26 isolates to eight commonly used fungicides and novel fungicide SYP-14288 were determined *in vitro*. The result shows that SYP-14288 has the strongest inhibitory activity on the mycelial growth of 26 isolates of *P. lythri*, with an average EC_{50} value of (0.33 ± 0.06) μg/mL, can be used as an effective fungicide for the control of strawberry tan-brown leaf spot disease. The average EC_{50} values were from 3.92 μg/mL to 72.58 μg/mL for epoxiconazole, difenoconazole, prochloraz, tebuconazole, chlorothalonil, and myclobutanil. Among them, azoxystrobin had lower toxicity, and mancozeb had the lowest inhibitory activity. The results of this study can provide an important reference for the rational use of fungicides to control strawberry tan-brown spot disease.

Keywords: *Pilidium lythri*; strawberry tan-brown leaf spot disease; mycelial growth; SYP-14288

草莓褐色叶斑病病原菌 *Pilidium lythri* 鉴定及室内防治药剂筛选

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摘 要：近年来，由病原菌 *Pilidium lythri* 引起的草莓褐色叶斑病，是一种在中国发生严重的新病害。作者在 2015 年至 2017 年间，从北京市昌平区、山东省诸城市和湖南省娄底市采集有症状的草莓样品共 90 株，每地 30 株，根据形态学特征、分子生物学及系统发育分析，其中 26 株分离物被鉴定为 *P. lythri*，并且山东和湖南系首次发现由 *P. lythri* 引起的草莓褐色叶斑病。鉴于目前中国还没有登记用于防治草莓褐色叶斑病的杀菌剂。本研究通过室内毒力测定法测定了 26 株 *P. lythri* 菌株对 8 种常用杀菌剂和 1 种新型杀菌剂双苯菌胺 (SYP-14288) 的敏感性。结果表明：SYP-14288 对 *P. lythri* 菌丝生长的抑制活性最强，平均 EC_{50} 值为 (0.33 ± 0.06) μg/mL，可将其作为防治草莓褐色叶斑病的首选药剂；氟环唑、苯醚甲环唑、咪鲜胺、戊唑醇、百菌清

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The authors declare that they have no competing interests.

和腈菌唑对 *P. lythri* 的平均 EC_{50} 值在 3.92~72.58 μg/mL 之间，其中菌株对嘧菌酯和代森锰锌的敏感性较低。该研究结果可为合理使用杀菌剂防治由 *P. lythri* 引起的草莓褐色叶斑病提供参考。

关键词: *Pilidium lythri*；草莓褐色叶斑病；菌丝生长；双苯菌胺
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0 Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is an important commercial fruit crop that is widely grown in temperate regions worldwide. Over the past twenty years, the planting area and yield of strawberry have grown rapidly. According to the latest statistics from FAOSTAT (https://www.fao.org/faostat/zh/#home) in 2020, strawberry planting areas have reached more than 100 000 hectares in China. Meanwhile, strawberry production was up to 3000 000 tons per year in China, ranking top of the world[1]. During the growth period, strawberries are affected by a variety of fungal pathogens, for example, *Botrytis cinerea* Pers., *Colletotrichum* spp., and *Verticillium dahliae* Kleb.[2]. *Pilidium lythri*, previously named *Pilidium concavum* and *Hainesia lythri*, has been reported to cause strawberry tan-brown rot disease worldwide. Strawberry tan-brown rot disease was first reported in Japan, and since then reported in several other countries such as Poland, Brazil, and Belgium[3-7]. In China, strawberry tan-brown rot disease caused by *P. lythri* has been reported only in Beijing so far[8].

In addition to strawberry, *P. lythri* can also infect more than ten hosts, including *Olea europaea*, *Fallopia japonica*, *Hieracium caespitosum*, *Bergenia crassifolia*, *Paeonia suffruticosa*, *Eucalyptus* sp., *Rosa rugosa*, *Prunus domestica*, *Paeonia lactiflora*, *Greyia radlkoferi*, *Aesculus hippocastanum*, *Vaccinium corymbosum* and *Cornus mas*[5-6,8-9]. *P. lythri* infects strawberry leaves, stems, fruits, and roots. After the initial acute infection, a circular, water-stained brown spot emerges in the center or margin of strawberry leaves[6-7].

The application of chemical fungicides is an effective method for managing strawberry diseases. However, the development of fungicide resistance has compromised this control strategy. Besides, considering the safety of fresh strawberry, there are also some limitations to the use of chemical fungicides in the field. Currently, the management of *P. lythri* heavily relies on the application of effective fungicides and host resistance. However, there is little research on the chemical control of strawberry tan-brown rot disease caused by *P. lythri*.

The purpose of this study is to identify and characterize *P. lythri* associated with tan-brown leaf spot disease of strawberry in China based on morphological, molecular and pathogenic characteristics and to evaluate the inhibitory activity of azoxystrobin, chlorothalonil, epoxiconazole, difenoconazole, mancozeb, myclobutanil, prochloraz, tebuconazole and SYP-14288 against this fungus *in vitro*.

1 Materials and methods

1.1 Medium

PDA：200 g potato, 20 g dextrose, and 15 g agar in 1000 mL distilled water.

1.2 Fungicides

Technical-grade azoxystrobin (95% a.i.; Syngenta Biotechnology Co., Ltd., Shanghai, China), chlorothalonil (98% a.i.; Henan Chunguang Agrochemicals Co., Ltd., Henan, China), difenoconazole (98% a.i.; Hangzhou Yulong Agrochemicals Co., Ltd., Zhejiang, China), epoxiconazole (97.8% a.i.; Henan Zhongzhou Seed Technology Development Co., Ltd., Henan, China), mancozeb (98.5% a.i.; Hebei Hesen Chemical Co., Ltd., Hebei, China), myclobutanil (98% a.i.; Henan Zhongyuan Germplasm Factory, Henan, China), prochloraz (97% a.i.; Hangzhou Qingfeng Agrochemicals Co., Ltd., Zhejiang, China), SYP-14288 (97% a.i.; Shenyang Research Institute of Chemical Industry, Shenyang, China), and tebuconazole (98% a.i.; Jiangsu Fengdeng Pesticide Co., Ltd., Jiangsu, China) were dissolved respectively in dimethyl sulfoxide (DMSO) to make stock solutions and were stored at 4 ℃ in the dark.

1.3 Diseased sample collection and pathogen isolation

A total of 90 diseased samples were collected randomly from strawberry fields exhibiting typical symptoms of tan-brown rot disease in Changping

District, Beijing; Zhucheng County, Shandong Province; and Loudi City, Hunan Province, China in 2015-2017, with 30 samples from each district. Among 26 isolates of *P. lythri*, one isolate (BJ-4) was from Beijing City, nine isolates (HNLD-1 to HNLD-7, HNLD-9, HNLD-12) were from Hunan Province, and 16 isolates (SD17-2 to SD17-12, SD14 to SD18) were from Shandong Province (Table 1). The infected

Table 1 Information of ITS and LSU gene sequence of *P. lythri* strains used for phylogenetic analysis

Species or strain	GenBank No. ITS	GenBank No. LSU	Host		Country	Reference
			Common name	Latin name		
P. lythri	AY487094.1	AY487095	Rose	*Rosa* sp.	USA	[11]
P. lythri	AY487097.1	AY487098	Peony	*Paeonia suffruticosa*	Japan	[11]
P. pseudoconcavum	KF777184.1	KF777236.1	Ash tree	*Greyia radlkoferi*	South Africa	[12]
P. acerinum	AY487088	AY487089	Horse chestnut	*Aesculus hippocastanum*	Netherlands	[11]
P. acerinum	AY487091.1	AY487092	garden soil	*Hortum terram*	Netherlands	[11]
P. eucalyptorum	KT950854.1	KT950868.1	Eucalyptus grandis	*Eucalyptus robusta*	France	[13]
Chaetomella raphigera	AY487076.1	AY487077	Blueberry	*Vaccinium corymbosum*	USA	[11]
BJ-4	MH322003	MH322002	Strawberry	*Fragaria* × *ananassa*	China	This study
HNLD-1	MH322005	MH322004	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-3	MH322016	MH322014	Strawberry	*Fragaria* × *ananassa*	China	This study
HNLD-2	MH322006	___	Strawberry	*Fragaria* × *ananassa*	China	This study
HNLD-3	MH322007	___	Strawberry	*Fragaria* × *ananassa*	China	This study
HNLD-4	MH322008	___	Strawberry	*Fragaria* × *ananassa*	China	This study
HNLD-5	MH322009	___	Strawberry	*Fragaria* × *ananassa*	China	This study
HNLD-6	MH322010	___	Strawberry	*Fragaria* × *ananassa*	China	This study
HNLD-7	MH322011	___	Strawberry	*Fragaria* × *ananassa*	China	This study
HNLD-9	MH322012	___	Strawberry	*Fragaria* × *ananassa*	China	This study
HNLD-12	MH322013	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-2	MH322015	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-4	MH322017	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-5	MH322018	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-6	MH322019	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-7	MH322020	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-8	MH322021	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-9	MH322022	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-10	MH322023	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-11	MH322024	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-12	MH322025	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-14	MH322026	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-15	MH322027	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-16	MH322028	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-17	MH322029	___	Strawberry	*Fragaria* × *ananassa*	China	This study
SD17-18	MH322030	___	Strawberry	*Fragaria* × *ananassa*	China	This study
P. lythri	JQ790789.1	___	Japanese knotweed	*Fallopia japonica*	USA	[14]
P. lythri	JX047867.1	___	Meadow hawkweed	*Hieracium caespitosum*	France	[15]
P. lythri	KF911079.1	___	Strawberry	*Fragaria* × *ananassa*	USA	[6]
P. lythri	JQ995228.1	___	Strawberry	*Fragaria* × *ananassa*	China	[8]
P. lythri	GU126750.1	___	Peony	*Paeonia suffruticosa*	China	[16]
P. lythri	FM211810.1	___	Chinese cabbage	*Bergenia crassifolia*	France	[17]
P. lythri	KJ908840.1	___	Strawberry	*Fragaria* × *ananassa*	Philippines	[18]
P. lythri	KF060281.1	___	Eucalyptus	*Eucalyptus* sp.	Mozambique	[19]
P. lythri	KF646103.1	___	Rugosa rose	*Rosa rugosa*	Lithuania	[20]
P. lythri	KC614564.1	___	European plum	*Prunus domestica*	Iran	[21]

Note: “___” indicates no LSU gene sequence.

parts were cut into pieces (0.5 cm in diameter) from the edges of lesions, soaked into sodium hypochlorite (1%, *V: V*) for 30 s, sterilized with 70% (*V: V*) ethanol for 60 s and washed with distilled water for 3 times with 10 s each time. The detached samples were dried on sterile paper and placed on PDA plates, and incubated at 25 ℃ in the dark for 7 days. Finally, the isolates were purified by single spores to obtain pure cultures[10] and transferred to PDA slants covered with sterile mineral oil for long-term storage under −20 ℃ at Beijing University of Agriculture.

1.4 Morphological description

The isolates were inoculated on PDA plates at 28 ℃ in the dark for 5 days. The shape and color of the colony, aerial mycelium, and conidium were recorded. For microscopic features, Riddell' slide culture techniques were used[22]. Each microstructure was measured 30 times and pictures of related properties were taken using an Olympus digital camera (DM-21) mounted on an optical microscope (Olympus BX-41, Olympus Optics).

1.5 Pathogenicity analysis

The pathogenicity of the isolates was verified by Koch's Principle[23]. Detached and untreated strawberry (cv. *Benihopp*) fruits and leaves were used as inoculation materials, which were cleaned with sterile water, surface disinfected with 0.8% (*V* : *V*) sodium hypochlorite for 2 minutes, rinsed with sterile water three times, and dried. Five leaves and fruits were inoculated with each isolate by placing the mycelial plugs (5 mm in diameter) that were cut with a cork borer from the margin of a five-day-old colony of isolates. Another five leaves and fruits were inoculated with 5-mm PDA plugs as the control group. After incubation in a chamber (Shanghai Yiheng Technical Co., Ltd., Shanghai, China) with 80% relative humidity, 12 h light/12 h darkness cycle at 28 ℃ for 5 days, *P. lythri* was reisolated as described above to confirm the pathogen identity. The experiment was performed twice.

1.6 Internal transcribed spacer (ITS) and large subunit (LSU) sequence analysis

Single-spore cultures developed on PDA medium were used for DNA extraction, using the rapid genome DNA extraction kit purchased from Beijing Biomed Biotechnology Co., Ltd. (Beijing, China). The DNA concentration of each isolate was determined by Nano-Drop ND-1000 Spectrophotometer (NanoDrop Technologies) in three replicates, and a suitable dilution was prepared. In the polymerase chain reaction (PCR), the partial regions of two loci, ITS rDNA and LSU rDNA, were amplified using the primers ITS5 (GGAAGTAAAAGTCGTAACAAGG) + ITS4 (TCCTCCGCTTATTGATATGC) [24] and LR0R (GTACCCGCTGAACTTAAGC) + LR7 (TACTACCACCAAGATCT) [25-26]. All PCRs are performed in a total volume of 25 μL, consisting of 50 ng genomic DNA, 1 × Dream Taq Green PCR Mastermix (Fermentas) and 0.2 μm of each primer. The LSU and ITS PCR conditions were described by de Gruyter et al. [27] and Woudenberg et al. [28], respectively. PCR products were confirmed by electrophoresis on a 1.0% (*V* : *V*) agarose gel in 1 × Tris-Borate-EDTA buffer and sequenced at Beijing Liuhe BGI Co., Ltd. (Beijing, China).

1.7 Phylogenetic analysis

The sequences of the isolates obtained in this study were spliced and compared by DNAMAN 6.0 (Lynnon Biosoft, USA). The assembled sequences were used as a query for BLAST (Mega BLAST from NCBI). The subjects with high similarity, including ex-type and ex-epitype samples were then downloaded from GenBank (Table 1). MrBayes Ver. 3.2.1 were used to build the Bayesian Inference (BI) phylogenetic trees for ITS and LSU genes amplified in the above isolates. The phylogenetic tree was rooted with *Chaetomella raphigera* voucher BPI 843541 (GenBank No. AY487077).

1.8 Fungicide sensitivity tests

Fungicide sensitivity was determined by the mycelial growth rate method *in vitro*[29]. Mycelial plugs (5 mm in diameter) were transferred from the leading edge of an actively growing colony onto a series of PDA plates amended with serial concentrations of azoxystrobin, chlorothalonil, difenoconazole, epoxiconazole, myclobutanil, mancozeb, prochloraz, SYP-14288, or tebuconazole (Table 2). DMSO-amended PDA was included in the control group. The final DMSO concentration in all plates in this study was adjusted to 0.1% (*V* : *V*), which did not affect the growth of *P. lythri*. After incubation at 25 ℃ in the dark for 8 days, the colony diameter was measured by cross method. Each treatment was replicated four times, and the experiment was conducted twice. The median effective concentration (EC_{50}) was calculated by

regressing the percentage of growth inhibition against the log fungicide concentration.

Table 2 Initial concentration and series dilution concentrations of fungicides

Fungicide	Series dilution concentrations/ ($\times 10^3$ μg/mL)
azoxystrobin	50, 100, 150, 200, 250, 300
chlorothalonil	5, 10, 20, 30, 40, 50
difenoconazole	1, 2, 3, 4, 5, 10
epoxiconazole	1, 2, 4, 6, 8, 10
myclobutanil	10, 25, 50, 75, 100, 150
mancozeb	10, 50, 100, 200, 500, 1000
prochloraz	1, 2, 4, 6, 8, 10
SYP-14288	0.1, 0.2, 0.3, 0.4, 0.5, 1.0
tebuconazole	1, 2, 4, 8, 16, 32

1.9 Data analysis

Statistical analyses were performed using the SAS package (SAS Institute, Cary, NC) Ver. 9.2. The EC_{50} values for each isolate were calculated by using PROC REG for linear regression. Mean values were compared using Fisher's least significant difference (LSD) test at a significance level of $\alpha = 0.05$.

2 Results and analysis

2.1 Morphology and cultural characteristics

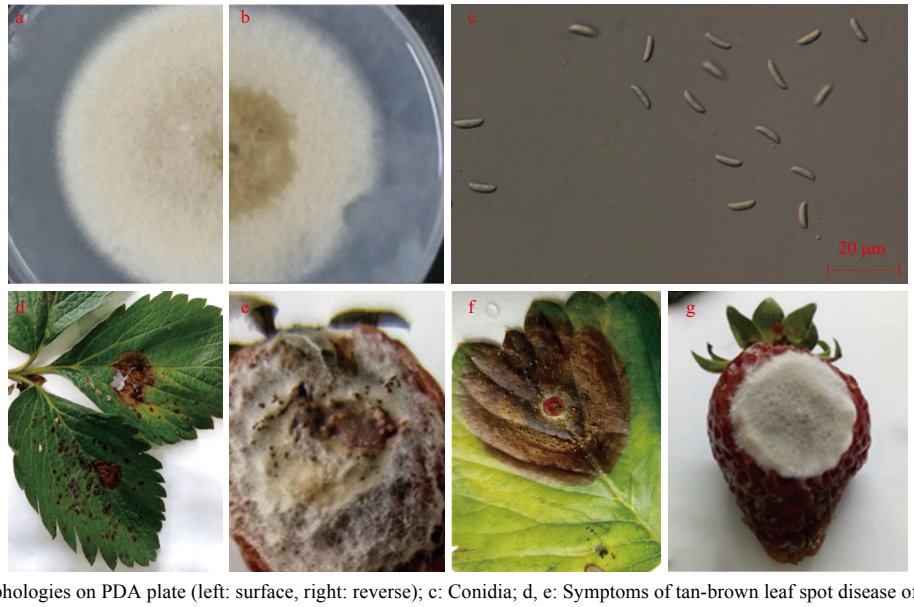
The colony of the tested isolates was circular with regular edges and exhibited white-to-cinnamon sparse aerial mycelium on PDA medium. In addition, a creamy-to-brown circle appeared around the center of the colony (Fig. 1 a, b). Conidia were unicellular, hyaline, smooth, and allantoid with a little bent pointing at both sides. The size of the conidia was 8-12 μm in length and 2.0-2.5 μm in width (Fig. 1 c). The sexual stage was not observed.

2.2 Pathogenicity analysis

Field symptoms of strawberry disease caused by *P. lythri* were similar to anthracnose. In the early stage of the disease, circular and brown spots are formed at the center or edge of the leaves. However, unlike anthracnose fruit rot with orange conidial masses, the fruit rotted by *P. lythri* produces pink conidial masses (Fig. 1 d, e). The 26 isolates were inoculated on healthy strawberry leaves and fruits. At the onset of infection, the spots on the margin or in the middle of leaves were very small, round, and water-soaked lesions. The spots enlarged gradually up to 1-3 cm in diameter, and the color ranged from brown to dark brown, with pink spore masses forming at the late stage (Fig. 1 f). On the fruit surface, white hyphae appeared at the early stage, and a white layer of mold formed at the late stage (Fig. 1 g). The pathogen was reisolated and purified from diseased leaves and fruits. The colony characteristics obtained were the same as those of the originally isolated pathogen.

2.3 Phylogenetic analysis

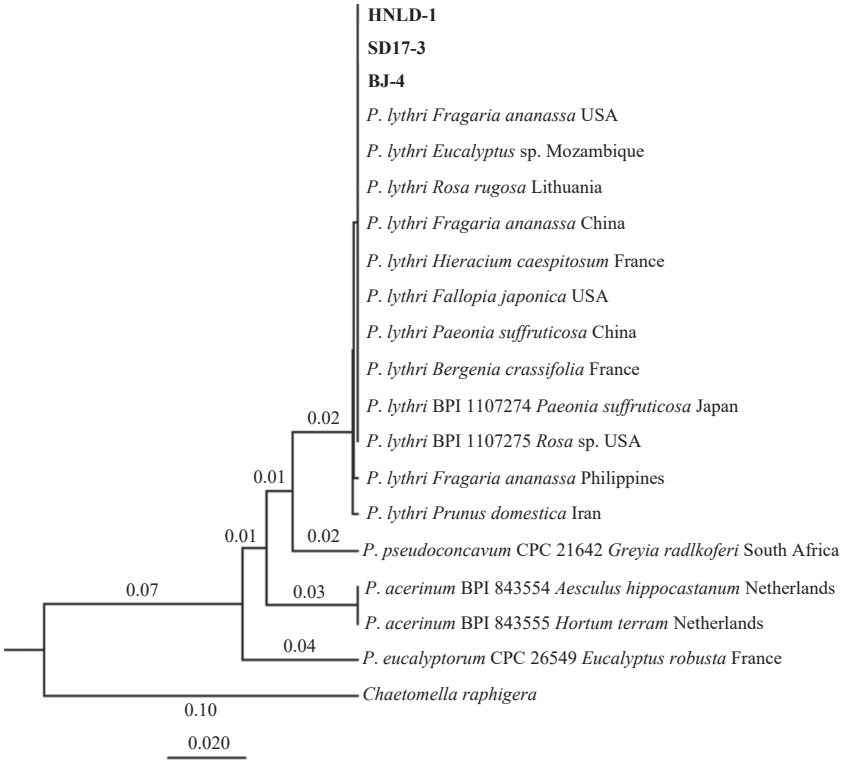
Three isolates were used for the phylogenetic analysis based on ITS and the concatenated ITS and LSU



a, b: Colony morphologies on PDA plate (left: surface, right: reverse); c: Conidia; d, e: Symptoms of tan-brown leaf spot disease of strawberry in the field; f, g: Pathogenicity tests on strawberry leaves(left) and fruits(right) using the isolated *P. lythri*. Scale bars = 20 μm.

Fig. 1 Tan-brown leaf spot disease and causal pathogen on strawberry

sequences. The final aligned ITS dataset contained 43 in-group taxa. All of the sequences were lodged in NCBI's GenBank nucleotide database (http://www.ncbi.nlm.nih.gov; Table 1). Only nine (*P. lythri* BJ-4, HNLD-1, SD17-3, and six control isolates) in group taxa (because most species recorded in GenBank losing LSU sequences) concatenated to form a supermatrix of 1193 bp (gene boundaries, ITS: 1-396 and LSU: 397-1, 193). The substitution model for ITS and LSU genes was analyzed using MrModeltest Ver. 2.3 with JC + G and HKY + G models, respectively. The ITS phylogeny grouped all examined Chinese isolates, collected from different hosts and geographical origins, in *P. lythri* clade with high posterior probability (Fig. 2). Through BI analysis, the concatenated sequences revealed again that distinct monophyletic groups probably contained *P. lythri* and *P. pseudoconcavum*. The group included *P. acerinum* and *P. eucalyptorum* was a sister group (Fig. 3). The results showed that the pathogen was *P. lythri* according to the morphological and phylogenetic analyses.



The phylogenetic tree was rooted with *C. raphigera* BPI 843541 and *Vaccinium corymbosum* USA. The scale bar shows 1.0 expected change per site. All isolates used for the phylogenetic analysis in this study are in bold type.

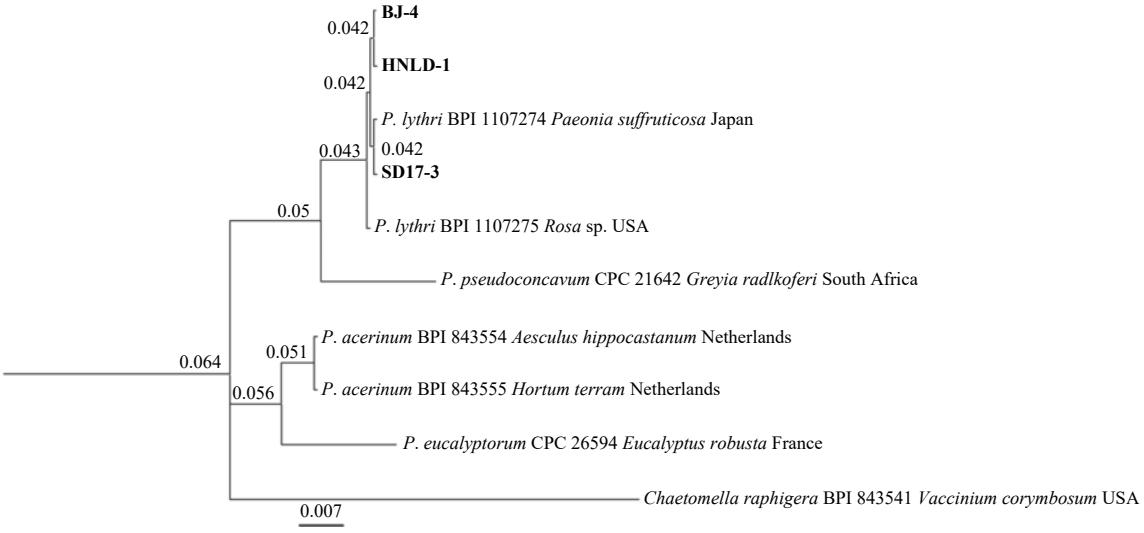
Fig. 2 ITS phylogenetic tree of *P. lythri* strains using Bayesian Inference based on the JC + G model

2.4 Fungicide sensitivity assessment *in vitro*

The sensitivities of the 26 *P. lythri* isolates to nine fungicides were tested as follows (Table 3). The results showed that SYP-14288 had the strongest inhibitory effect on the mycelial growth of *P. lythri*, with an average EC_{50} value of (0.33 ± 0.06) μg/mL, which indicated that it could be used as an effective fungicide for controlling strawberry brown spot disease. Epoxiconazole, difenoconazole, prochloraz, and tebuconazole had better inhibitory effects and *P. lythri* was relatively less sensitive to chlorothalonil, myclobutanil, and azoxystrobin. Mancozeb had the lowest toxicity, with an average EC_{50} value of (336.16 ± 158.21) μg/mL.

3 Discussion

Among *Pilidium* spp., *P. lythri* has been the most reported cause of plant diseases. *P. lythri* is a newly discovered strawberry tan-brown leaf spot disease in China[8]. In this study, 26 isolates were isolated from strawberry tissues in China, morphological characterization of the isolates was completely consistent with the descriptions of *P. lythri* (synanamorph *P. concavum* and *H. lythri*) [11-13, 30]. However, the sexual morph of *P. lythri* described as *D. oenotherae* (Cooke & Ellis) Nannf. [31] was not observed. Nannfeldt (1932) placed



The phylogenetic tree was rooted with *C. raphigera* BPI 843541 and *V. corymbosum* USA. The scale bar shows 0.1 expected changes per site. The strawberry isolates used for phylogenetic analysis in this study are in bold type.

Fig. 3 ITS and LSU phylogenetic tree of *P. lythri*, isolates using Bayesian Inference based on the models of JC + G and HKY + G

Table 3 Sensitivity of *P. lythri* isolates to nine fungicides *in vitro*

Fungicide	EC_{50} value/(μg/mL)		
	Minimum	Maximum	Mean value*
azoxystrobin	46.01	561.57	157.09 ± 25.06 e
chlorothalonil	6.32	110.26	27.72 ± 4.57 c
epoxiconazole	1.82	8.89	3.92 ± 0.34 ab
difenoconazole	2.59	8.33	4.84 ± 0.30 ab
mancozeb	44.63	871.77	336.16 ± 158.21 f
myclobutanil	55.31	100.79	72.58 ± 2.10 d
prochloraz	1.57	10.87	5.37 ± 0.44 ab
SYP-14288	0.11	0.62	0.33 ± 0.06 a
tebuconazole	2.12	21.91	9.18 ± 0.87 b

Note: * Same letters after the data in the same column are not significantly different by Fisher's least significance difference ($\alpha = 0.05$).

this teleomorph in the monotypic genus *Discohainesia* Nannf. as a member of the Dermateaceae, Leotiales[31]. However, living cultures derived from ascospores do not exist. Palm[30] examined numerous specimens and provided a detailed description including growth characteristics for this species. The teleomorph is relatively uncommon and inconspicuous. Only sporodochia were formed on media tested and pycnidia were absent, in agreement with previous reports of *Pilidium* rot on strawberries[7]. Therefore, morphological identification was made only by mycelium and conidial characteristics. Moreover, 26 isolates were identified as *P. lythri* by phylogenetic analysis using ITS rDNA individually and concatenated combined datasets of ITS and LSU rDNA (Fig. 2 and Fig. 3). In China, the strawberry tan-brown spot disease caused by *P. lythri* was first discovered in a greenhouse in Xingshou Town, Changping District, Beijing City[8], and this is the first report of *P. lythri* caused tan-brown spot disease on strawberries in Zhucheng County, Shandong Province; and Loudi City, Hunan Province.

Currently, fungicides registered for the control of strawberry fungal diseases in China including azoxystrobin (quinone outside inhibitor, QoI), trifloxystrobin (QoI), difenoconazole (triazole), tebuconazole (triazole), and fluopyram (succinate dehydrogenase inhibitor, SDHI)[32]. However, there are no fungicides registered to control tan-brown spot disease on strawberries caused by *P. lythri*. Furthermore, the symptoms of this disease in the field are very similar to strawberry anthracnose. The symptoms of these two diseases can easily be misinterpreted, which results in drug misuse and delay of the proper period of prevention and control of tan-brown spot disease on strawberries. Therefore, the control efficacy is not ideal.

In our study, three fungicides registered for controlling fungal diseases on strawberries (azoxystrobin, difenoconazole, and tebuconazole), five commonly used fungicides with different mechanisms and models of action, and SYP-14288 were selected as the test fungicides. The sensitivity of these nine fungicides on strawberries was determined by the mycelial growth rate method[29]. SYP-14288 is

a novel compound developed by the China Shenyang Research Institute of Chemical Industry, which is an environmentally friendly fungicide with low toxicity to mammals (unpublished data). SYP-14288 is highly effective against 32 important plant pathogens belonging to a range of taxonomic groups *in vitro* and acts as an uncoupler of oxidative phosphorylation[33]. Similar to the chemical structure of fluazinam, SYP-14288 is also a dinitroaniline compound, but its production cost is only one-third of that of fluazinam, and its activity against oomycetes is better than that of fluazinam[34]. However, this product has not yet been registered in China. Our results showed that SYP-14288 has a strong inhibitory effect on the mycelial growth of 26 isolates of *P. lythri*. Previous studies[35] have shown that SYP-14288 has excellent antimicrobial activity on the spore germination of tested pathogens including oomycetes, ascomycetes, conjugated fungi, and asexual fungi, and the antifungal activity on the spore germination stage of the same pathogen is generally higher than that on the mycelial growth. The effect of this agent on the spore production and mycelial growth of *P. lythri* can be further explored in the future. The inhibition activity of mancozeb was the lowest, which was consistent with the results that the fungicidal activities of SYP-14288 against *B. cinerea*, *C. orbiculare*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Alternaria alternata*, and *Macrophoma kawatsukai* were higher than those of chlorothalonil and mancozeb[36]. These results indicated that SYP-14288 could be used as an effective fungicide for the control of tan-brown spot disease on strawberries.

The multiple of the maximum EC_{50} value and the minimum EC_{50} value of azoxystrobin, chlorothalonil, mancozeb, and tebuconazole was higher than 10 times. It has been speculated that the isolates have developed resistance to these fungicides. As reported, azoxystrobin, chlorothalonil, and mancozeb have been widely used to control strawberry fungal diseases in China, such as strawberry root rot disease, anthracnose, grey mold, and powdery mildew[37]. A single fungicide used extensively for a long time can easily cause resistance. Therefore, we suggest the alternative use of different fungicides that have different mechanisms and models of action to control tan-brown spot disease on strawberries, such as the application of SYP-14288 alternately with QoI, DMI, and SDHI fungicides. This study was carried out only *in vitro*, and could not represent the actual application effect of the fungicides in the field, where the mechanism of action, the inner-absorption conducting properties, and other factors may affect the efficacy of practical application. Therefore, in order to accurately guide the control of strawberry disease, field experiments need to be further verified and the biocides be applied alternately.

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