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ORIGINAL PAPER

Antifungal substances produced by *Penicillium oxalicum* strain PY-1—potential antibiotics against plant pathogenic fungi

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Abstract This paper reports the isolation from soil of *Penicillium* strain PY-1 with strong antagonistic activity against plant pathogenic fungi. On the basis of its morphological characteristics and the sequence of the ITS region, strain PY-1 was identified as *P. oxalicum*. Strain PY-1 produces antifungal substances that suppress the mycelial growth of *Sclerotinia sclerotiorum* and many other plant pathogenic fungi tested; the highest antagonistic activity was detected at 72 h when cultured in a 250-ml flask containing 80 ml potato dextrose broth. Compared with carbendazim, the relative activity of the antifungal substances produced by strain PY-1 was approximately 4 μg active ingredient (a.i.) per milliliter. The antifungal substances were extracted with ethyl acetate and further separated by high-performance liquid chromatography (HPLC); at least two active components were discovered. The ability to control plant disease with strain PY-1 was confirmed with *S. sclerotiorum*, a widespread pathogenic fungus that attacks rapeseed (*Brassica napus*) and other plants. Spores (10^6 or 10^7 ml^{-1}) and filtrate (tenfold diluted or undiluted) of strain PY-1 could significantly suppress infection and/or the extent of infection by *S. sclerotiorum* of plants at seven-true-leaves stage. The potential of strain PY-1 for identifying new antibiotics to control fungal disease and for biological control of plant disease, for example oilseed rape stem rot, is discussed.

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Introduction

Fungal plant pathogens, which infect all major crops, are a threat to global food security; they cause serious losses both in the field and post-harvest, and some may produce mycotoxins (Strange and Scott 2005). Various artificial chemical fungicides have been successfully developed and applied to fields to control fungal diseases. However, because of their huge populations and high frequency of mutation, pathogenic fungi may easily acquire resistance to frequently used fungicides. Several important chemical fungicides, for example anilinopyrimidine, benzimidazoles, demethylation inhibitors (DMI), dicarboximide, phenylpyrrole, Qo respiration inhibitors, and strobilurin, have lost high efficacy against pathogenic fungi in the field (LaMondia and Douglas 1997; Shi et al. 2000; Baroffio et al. 2003; McGrath and Shishkoff 2003; Yuan and Zhou 2004; Sallato and Latorre 2006). To reduce the risk of crop disease control and enhance the safety of food and the environment, new safe fungicides should be discovered and developed. The skeletal structures of new antifungal compounds usually come from natural sources (Bevan et al. 1995). This involves screening of microorganisms and plant extracts, using a variety of models (Shadomy 1987). Today, the emphasis is on the exploration of unusual and previously ignored ecosystems, using a variety of selected novel targets (Gagg et al. 1997; Von Dohren and Grafe 1997; Hegde et al. 2001; Phoebe et al. 2001). Secondary metabolites produced by microorganisms are the main source of the skeletons; some have been used directly

in field; Jinggangmycin, for example, has been used to control rice sheath blight (*Rhizoctonia solani*) in China for more than 30 years.

Sclerotinia sclerotiorum is a plant pathogenic fungus found inhabiting soil ubiquitously in many parts of the world. It attacks more than 450 species and subspecies among 64 genera of plants (Boland and Hall 1992). In China, this pathogen causes stem rot on oilseed rape (*Brassica napus*), and results in serious losses every year in the middle and low drainage areas of the Yangtse River. *S. sclerotiorum* also damages vegetable crops, for example lettuce, celery, and beans, during spring and early summer, and other oil crops such as sunflower (*Helianthus annuus* L.) and soybean (*Glycine max* (L.) Merr) in North China. To control this pathogenic fungus, chemical fungicides, for example carbendazol, are applied to fields during the blooming period. The efficacy has declined dramatically in recent years, however, and a fungicide-resistant strain has frequently been isolated from fields (Shi et al. 2000). To deal with the risk of an epidemic of chemical fungicide-resistant strains in crop fields, it is important to screen new antifungal substances against *S. sclerotiorum*. In this paper we describe an antifungal substance-producing *Penicillium* fungus strain PY-1 and the primary characteristics of the antifungal substance.

Materials and methods

Microorganism, medium and culture conditions

Penicillium fungal strain PY-1 was originally isolated from soil sampled from Zhejiang Province, PR China. Plant pathogenic fungi tested in this study were supplied by CCAM (Collection Centre of the State Key Lab of Agriculture Microbiology). Stock cultures were maintained on potato dextrose agar (PDA) slant (200 g peeled potato/l, 20 g dextrose/l, and 18 g agar/l; PDB, potato dextrose broth) and stored at 2–6°C. *Penicillium* strain PY-1 and pathogenic fungi except *Sclerotinina* species were cultured on PDA plates and incubated at 25 ± 1°C; *S. sclerotiorum* and other species in the genus *Sclerotinia* were incubated at 20 ± 2°C.

Identification of strain PY-1

The *Penicillium* strain PY-1 was identified by two methods. One was based on colony morphology, development and shape of conidiophores, phialide arrangement, spore shape, and arrangement when strain PY-1 grew on a PDA plate; the other was based on the internal transcribed spacer (ITS) region sequence. To extract genomic DNA, spores of strain PY-1 were spread on a cellophane membrane, by placing the membrane on the PDA plate and incubating for 36 h. Mycelia were then harvested by removing the mycelial membrane formed on the cellophane membrane and genomic DNA was extracted with CTAB by following the standard procedure (Sambrook and Russell 2000). The ITS DNA was amplified by PCR with two universal primers specific to fungi, ITS1 (5′TCC GTA GGT GAA CCT GCG G 3′) and ITS4 (5′TCC TCC GCT TAT TGA TAT GC 3′), designed by White et al. (1990). The PCR product was ligated into pUCm-T vector (TAKARA, Dalian, PR China) and transformed into *E. coli* JM109. Automatic sequencing was carried out with BigDye kit and ABI program for two positive clones. The sequence of strain PY-1 was subjected to a homology search using the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/).

Preparation of fungal inoculum

To produce spores of *Penicillium* strain PY-1, 2 ml sterile distilled water was added to a 10-day culture of strain PY-1 on a PDA slant, a spore-suspension was made with a sterile glass rod, and then 200 µl spore suspension (10^7 spores ml^{-1}) was transferred evenly to a fresh PDA plate (ϕ 90 mm). After incubation at 25°C for 1 week, spores were collected by washing the colony with 15 ml sterile distilled water. After removal of mycelial debris, by passage through three layers of cheesecloth, the spore suspension was diluted serially and the concentration was counted with a hemacytometer. To prepare *S. sclerotiorum* and other species in the genus *Sclerotinia*, mycelial agar plugs were transferred from a PDA slant to fresh PDA plates and incubated at room temperature for activation; agar plugs from the colony were then removed to a fresh PDA plate for 2–5 days, based on the growth of pathogens, and were used as inocula.

To obtain culture filtrate, 500 µl spore suspension ($\sim 10^7$ spores ml^{-1}) was inoculated into a 250-ml flask containing 80 ml home-made potato dextrose broth; this was then shaken (180 rpm) at 25°C for 3 days. Filtrate was collected, using three layers of cheesecloth to remove the mycelial mass, incubated at 50°C for 10 min to kill active spores and hyphae, then stored at 2–6°C before use.

In-vitro antagonism test

Four mycelial plugs (φ 6 mm) of *S. sclerotiorum* or other plant pathogenic fungi cut from actively growing margins were placed equidistant from each other on a fresh Petri dish (φ 90 mm) containing 20 ml PDA, approximately 10 mm from the wall of the dish. Agar plugs of strain PY-1 were cut from a 10-day-old PDA culture and placed at the center of the pathogen-inoculated plate. PDA plates inoculated with pathogen plugs only were used as control

plates. The diameters of inhibition zones were measured after the colonies in the control plates filled the dishes. Each test was replicated three times.

In-vivo inhibition of *S. sclerotiorum* mycelial infection

Spores and culture filtrate of strain PY-1 were both used to check the inhibition ability of *S. sclerotiorum* infection. Spores and liquid culture filtrate were collected as mentioned above. Spore suspension was diluted to 10^7 and 10^6 spores ml^{-1}; a three-day-culture filtrate collected from the fermented liquid, or tenfold dilution, was used directly.

A hyphae-mediated infection technique was used to evaluate the efficacy of spores and filtrate at inhibition of infection by *S. sclerotiorum* of oilseed rape (*B. napus* L.), cv Huaza No.3. Seedlings of oilseed rape were planted in pots, with two seedlings sharing one pot, and plants with seven true leaves were used for the test. To prepare the inocula of pathogen, three mycelial agar disks (6 mm in diameter) cut from active colony edges were inoculated into a 250-ml flask containing 80 ml PDB and incubated in a shaker at a speed of 180 rpm at 20°C for 3 days. The mycelial mass was collected using sterilized cheesecloth, rinsed with sterile water to remove residual nutrients, then homogenized in a small sterile blender with sterile water; most hyphae fragments were approximately 100–200 μm long. The hyphae fragments were collected by centrifugation (3,000*g*) for 10 min and re-suspended in sterile water at 10^4 fragments ml^{-1}. Spores, culture filtrate of strain PY-1, or sterile distilled water were sprayed on the plants to wetness. After air-drying, hyphae fragments of the pathogen were sprayed on the same plant, again to wetness. The inoculated plants were moved to an incubator at 20 ± 2°C kept 100% relative humility for 1 week with 12 h fluorescence light each day. Both number and size of lesions caused by *S. sclerotiorum* were noted. There were four replicates for each treatment.

Primary extraction of antifungal substance

To investigate the optimum time for collection of antifungal substance, 0.5 ml spore-suspension containing 10^7 spores ml^{-1} was inoculated into a 250-ml flask containing 80 ml PDB, and shaken at 25°C at a speed of 180 rpm. Every 24 h after inoculation, 1.8 ml liquid was sampled and then passed through a filter membrane (ϕ 0.22 μm) to remove hyphae and spores. The filtrate (0.5 ml) was mixed with 9.5 ml warm PDA (ϕ 90 mm) and then poured into a sterilized Petri dish (ϕ 6.0 cm). Each treatment was replicated three times. Plates were incubated with an active mycelial agar plug of *S. sclerotiorum* at 20°C for 48 h and the diameter of the colony was then measured. Sterile distilled water (0.5 ml) was used as control. The diameters of colonies of *S. sclerotiorum* treated with filtrate and with water control were denoted *d*t and *d*c, respectively. The extent of suppression was calculated by use of the formula: $S(\%) = (dc - dt)/dc \times 100$. This experiment was repeated three times under the same conditions.

To test the relative activity of antifungal substance produced, a common fungicide, carbendazim was used as a standard. Carbendazim was dissolved in water; after passage through a filter (0.22 μm) the liquid was diluted to 100 μg a.i./ml and then serially diluted 10–100-fold. One milliliter of liquid was added to 9 ml warm PDA to prepare fungicide-containing plates of different concentration, and the plates were then inoculated with an active mycelial agar plug of *S. sclerotiorum* as described above. Suppression by different concentrations of carbendazim was compared with that by filtrate of strain PY-1.

To isolate antifungal substances, 100 ml culture filtrate was extracted with the same volume of ethyl acetate. The organic phase was concentrated by rotary evaporation (RE-52A, Henangongyi, China) at 42°C. The concentrated product was regarded as a crude sample and kept at −20°C. To check its antifungal activity the crude sample was dissolved in 10 ml methanol, and 50 μl samples were then loaded on to sterile filter paper discs (ϕ 8 mm). These loaded filter paper discs were placed at the center of *Petri* dishes containing PDA and inoculated with mycelial plugs of *S. sclerotiorum.* When the hyphae filled the control plates the inhibition zones were recorded for all treatments.

Primary purification of antifungal substance

Crude sample was separated by thin-layer chromatography (TLC). To locate the active band, a polyester sheet was cut into strips 5 mm wide and laid on a PDA plate. Active mycelial agar discs of *S. sclerotiorum* were placed on the two sides of the strips, parallel to the strips, at a distance of approximately 2.0 mm, and the plate was then incubated at 20°C for 36 h. The active band on the thin sheet was collected and re-extracted with ethyl acetate.

To further separate and purify the antifungal substance, high-performance liquid chromatography (HPLC) was used. A 4.6 × 150 mm, 5-μm particle, C_{18} reversed-phase column (YWG C_{18}, DICP, China) was used in a Shimadzu (Japan) LC-10AT chromatograph. The mobile phase for the first run was a gradient from 40–90% methanol in double-distilled H_2O over 50 min. The flow rate was set to 1.0 ml min^{-1}. When the crude sample was mainly separated, the central peak was collected in clean glass tubes. To check its activity, the method described above was used after evaporation of the solution in methanol and

double-distilled water to dryness and re-dissolution of the residue in a small amount of ether. The elute containing antifungal activity was further separated by HPLC with 70% methanol–30% double-distilled H_2O as mobile phase at a flow rate of 0.8 ml min^{-1}.

Data analysis

Data from completely randomized experiments were subjected to analysis of variance (ANOVA) using SAS software. Treatment means were compared with least significant difference (LSD) at a probability of 5%. Percentage suppression was angularly transformed before calculating mean values.

Results

Identification of *Penicillium* strain PY-1

Strain PY-1 has a low growth rate on PDA (~1.0 mm/day), and produces olive green conidia covering the colony surface at a late stage of growth. However, no deep pigment could be observed in the agar medium. Conidiophores developed in the colony individually and branched asymmetrically, with several phialides (often 6–10). Conidiosphores and conidia (4.7 ± 1.3 × 3.5 ± 0.6 μm) were budded from the phialids. Conidia on phialides were arranged as chains, and formed a column-like structure when several conidia chains congregated together. A DNA fragment of size 590 bp was amplified with fungal universal PCR primers ITS1 and ITS4. The sequence of the amplified DNA contained ITS 1, 5.8 S rDNA, and ITS 2, and showed 100% homology with the ITS sequence of *Penicllium oxalicum* (Peterson 2000).

In-vitro antagonism by *Penicillium* strain PY-1

Strain PY-1 strongly inhibited mycelial growth of *S. sclerotiorum* on PDA plates. The inhibition zone could remain dormant for more than 20 days, and at this time hyphae of *S. sclerotiorum* around the inhibition zone turned brown and died, with few sclerotia produced (Fig. 1); the diameter of the inhibition zone formed was 3.3 ± 0.26 cm. Strain PY-1 also strongly inhibited other species in the genus *Sclerotinia*, and other plant pathogenic fungi (Table 1).

In-vivo inhibition of *S. sclerotiorum* mycelial infection

Both spore and culture filtrate of strain PY-1 suppressed infection of oilseed rape leaves by *S. sclerotiorum* and

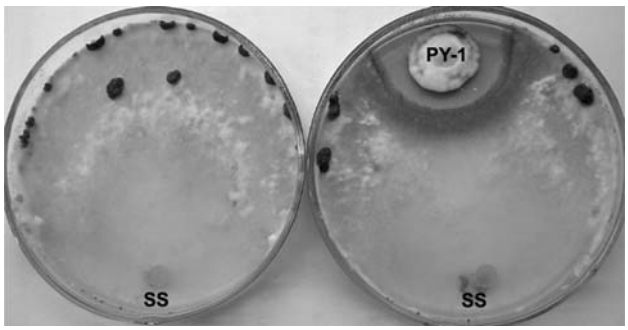

Fig. 1 Inhibition of the growth of *S. sclerotium* (SS) by *P. oxalicum* strain PY-1 cultured on a PDA plate at 20–22°C for 1 week: *left*, control; *right*, in the presence of *P. oxalicum*

successfully reduce the size of lesions. Efficacy was significantly dependent on the concentration of spores or culture filtrate. On application of 10^7 spores ml^{-1} to plants, no visible lesion could be seen during 1 week. When 10^6 spores ml^{-1} were applied, some leaves were infected. The average number of lesions on each pot was 2.0 ± 0.71, significantly lower than the 6.5 ± 0.5 for the water-treated control. The average diameter of lesions after spore-treatment was also significantly smaller ($\alpha = 0.05$), at 1.47 ± 0.2 cm (Table 2).

Both undiluted filtrate and tenfold-diluted filtrate could suppress lesion formation and reduce the size of lesions compared with water control. No detectable lesion was produced on plants treated with undiluted liquid. When plants were treated with tenfold-diluted filtrate lesions were formed at an average size of 1.39 ± 0.11 cm or completely suppressed (Table 2).

Accumulation of antifungal substance over time in liquid culture

Samples taken from liquid culture at 24-h intervals were analyzed for accumulation of the antifungal substance produced in PDB in a 250-ml flask. Antifungal activity increased with time; weak activity could be seen at 24-h and then rapidly reached a peak on the 3rd day. Suppression on the 3rd day was 77%; activity slowly decreased after the 4th day, however (Fig. 2).

Compared with carbendazim, the relative activity of antifungal substance of strain PY-1 produced at the 3rd day was about 4 μg a.i. per ml.

Primary purification of the antifungal substance

When the mobile phase was 75% methanol and 25% double-distilled water at a flow rate of 1.0 ml min^{-1} antifungal activity was detected from 6–8 min, with maximum

Table 1 Antagonism of *Penicillium oxalicum* strain PY-1 against selected plant pathogenic fungi[a]

Plant pathogenic fungi	Strain	Disease caused	Original host and location	Inhibition zone (cm diameter)
Sclerotinia sclerotiorum	SUN-F-M	Watery soft rot, cottony soft rot, drop, mold	Sunflower (*Helianthus tuberose*); Huhehaote, China	3.3 ± 0.26
S. minor	3-4-1	Watery soft rot, cottony soft rot, drop, mold	Lettuce (*Lactuca sativa*); Shennongjia, China	3.1 ± 0.16
S. nivalis	Let-19	Usually attacks lettuce and causes drop	Lettuce (*L. sativa*); Shennongjia, China	3.1 ± 0.05
S. trifoliorum	Ywd-A	Rot, white mold	Wild vetch (*Vicia* sp.); Wuhan, China	3.3 ± 0.08
Alternaria alternata	YS-1	Black spot on leaf	Chinese atractylodes (*Atractylodes lances*); Yingshan, China	4.0 ± 0.05
Bipolaris maydis	SLB-5	Southern leaf blight	Corn (*Zea mays*), Wuhan, China	3.9 ± 0.12
Botrytis cinerea	SB-1	Grey mold, rot	Strawberry (*Fragaria ananassa*), Wuhan, China	3.6 ± 0.09
Colletotrichum gloeosporioides	WHCGl-1	Anthracnose	Cotton (*Gossypium hirsutum*), Wuhan, China	3.9 ± 0.17
Fusarium oxysporum f.sp. *vasinfectum*	CFW-3	Fusarium wilt	Cotton (*G. hirsutum*), Wuhan, China	3.7 ± 0.05
Gibberella zeae	XG-12	Wheat scab	Wheat (*Triticum aestivum*), Xiaogan, China	3.6 ± 0.41
Magnaporthe grisea	MG-A	Rice blast	Rice (*Oryza sativa*), Anshi, China	3.5 ± 0.12
Monilinia laxa	ML-2	Brown rot of peach	Peach (*Prunus armeniaca*), Wuhan, China	3.3 ± 0.05
Pestalotiopsis sp.	GJ-1	Leaf spot	Gardenia (*Gardenia jasminoides*), Changyang, China	3.2 ± 0.14
Verticillium dahilae	CVW-4	Verticillium wilt	Cotton (*G. hirsutum*), Jingzhou, China	3.9 ± 0.16
Rhizoctonia solani AG-1 IA	WH-1	Rice sheath blight	Rice (O. sativa); Wuhan, China	NE[b]
Sclerotium rolfsii	CSB-1	Southern sclerotium blight	Cucumber (*Cucumis sativus*); Wuhan, China	NE

[a] The size of inhibition was measured when the four colonies in control covered the whole plate

[b] No effect

activity at a retention time of 6.853 min (Fig. 3). Eluate with antifungal activity from the first separation was subjected to a second separation with 70% methanol and 30% double-distilled water as mobile phase at a flow rate of 0.8 ml min^{-1}; two peaks with activity were detected at retention times 11.04 and 13.60 min (Fig. 3). This indicates the antifungal substance produced by PY-1 contains at least two active components.

Discussion

An antifungal substance produced by *Peniciliium* strain PY-1 effectively suppresses the growth of, and infection by, *S. sclerotiorum*. On the basis of morphology and the ITS sequence, strain PY-1 was identified as *Penicillium oxalicum*. As a biological control agent, *P. oxalicum* could suppress several plant diseases, for example *Phytophthora* root rot of azalea (Gintis and Benson 1987), *Pythium* seed rot and pre-emergence damping-off of chickpea (Trapero-Casas et al. 1990), and tomato *Fusarium* wilt and *Verticillium* wilt (De Cal et al. 1995, 1997, 1999; Larena et al. 2003; Sabuquillo et al. 2005). Evidence suggested that the biocontrol mechanism of *P. oxalicum* is mainly because of induced resistance of the plant (De Cal et al. 2000); Pectinases from *P. oxalicum* could induce resistance in cucumber against *Cladosporium cucumerinum* (Peng et al. 2004). *P. oxalicum* also has the ability to improve soil nutrition, by producing acid substances which solubilize barely soluble phosphates, subsequently improving growth

Table 2 Effect of *Penicillium oxalicum* strain PY-1 in suppressing lesion formation and expansion caused by *Sclerotinia sclerotiorum* on plants of oilseed rape (*Brassica napus*) with seven true leaves[a]

Treatment	Lesions induced per pot	Diameter of lesions (cm)
CK	6.5 ± 0.50	2.80 ± 0.50
10^7 spores	ND[b]	
10^6 spores	2.0 ± 0.71	1.47 ± 0.20
Undiluted filtrate	ND	
Tenfold diluted filtrate	4.0 ± 0.71	1.39 ± 0.11
E.D.F	15	15
LSD (P = 0.05)	1.01	0.88

[a] Hyphae fragments of *S. sclerotiorum* were used as pathogenic inoculum. Inoculated plants were moved for 1 week to a chamber at 20–22°C with 100% relative humidity and 12 h fluorescence light each day

[b] No detectable lesion induced in this treatment

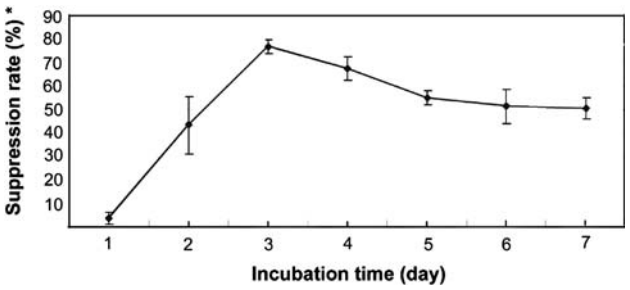

Fig. 2 The time course of antifungal activity produced by *Penicillium oxalicum* strain PY-1 over time. A 0.5-ml sample containing 10^7 spores ml^{-1} was inoculated into a 250-ml flask containing 80 ml PDB and shaken at 25°C at a speed of 180 rpm. *Suppression rate was calculated by use of the formula $S(\%) = (dc - dt)/dc \times 100$ (see main text for details) and then angularly transformed

of crops (Klimek-Ochab et al. 2003; Shin et al. 2005). As far as we are aware this is the first report of *P. oxalicum* producing an antifungal substance containing least two active compounds.

Fungi of the genus *Penicillium* are important producers of antibacterial compounds, but only a few are reported to produce antifungal substances (Kumagai et al. 1990; Matsukuma et al. 1992; Jackson et al. 1993; Okada et al. 1998; Nose et al. 2000; Sasaki et al. 2000; Kaiserer et al. 2003; He et al. 2004; Nicoletti et al. 2004). HPLC separation results showed that strain PY-1 produced at least two active compounds which may significantly inhibit mycelial growth of many pathogenic fungi and suppress infection by *S. sclerotiorum* when aerially applied. It is necessary to determine the chemical structures of the antifungal substances produced by strain PY-1.

Several strains of *P. oxalicum* have been reported as plant pathogens causing both blue eye mold on corn stored

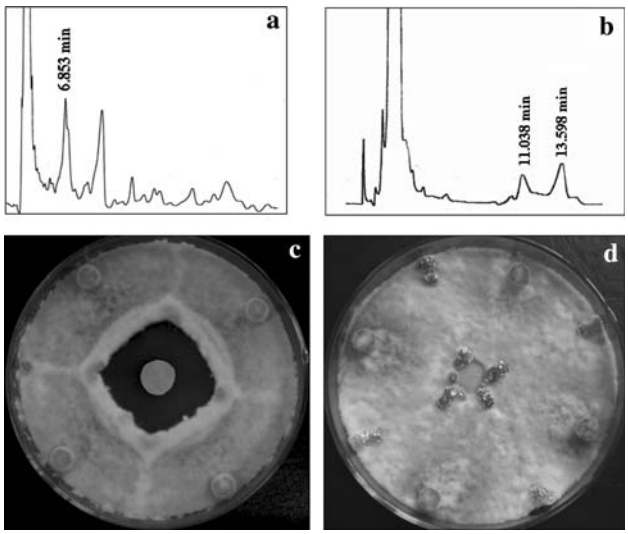

Fig. 3 The antifungal substance produced by *P. oxalicum* strain PY-1 was isolated by high-performance liquid chromatography (HPLC). (**a**) The first separation furnished one peak with activity, at a retention time of 6.853 min. (**b**) The second separation furnished two peaks with activity, at retention times of 11.038 min and 13.598 min. (**c**) Samples collected on elution of peaks with activity suppressed mycelial growth of *S. sclerotiorum*. (**d**) Samples collected on elution of peaks without activity did not suppress the mycelial growth of *S. sclerotiorum*. The fungus tested was cultured on a PDA plate for 5 days

on the cob in cribs and *Penicillium* stem rot of cucumber (Jarvis et al. 1990). Some strains have been reported to produce the mycotoxin secalonic acid D (Reddy et al. 1981; Balasubramanian et al. 2000). In our test, strain PY-1 did not cause lesions on the stem and leaves of oilseed rape, cucumber, and corn when these were inoculated with spores; we did not, however, check whether or not strain PY-1 could produce mycotoxin. To avoid potential health problems provoked by mycotoxin, strain PY-1 should be carefully examined.

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References

Balasubramanian G, Hanumegowda U, Reddy CS (2000) Secalonic acid D alters the nature of and inhibits the binding of the transcription factors to the phorbol 12-O-tetradecanoate-13 acetate-response element in the developing murine secondary palate. Toxicol Appl Pharmacol 169:142–150

Baroffio CA, Siegfried W, Hilber UW (2003) Long-term monitoring for resistance of *Botryotinia fuckeliana* to anilinopyrimidine, phenylpyrrole, and hydroxyanilide fungicides in Switzerland. Plant Dis 87:662–666

Bevan P, Ryder H, Shaw I (1995) Identifying small-molecule lead compounds: the screening approach to drug discovery. Trends Biotechnol 13:115–121

Boland GF, Hall R (1994) An index of plant hosts susceptible to *Sclerotinia sclerotiorum*. Can J Plant Pathol 16:93–108

De Cal A, Pascual S, Larena I, Melgarejo P (1995) Biological control of *Fusarium oxysporum* f. sp. *lycopersici*. Plant Pathol 44:909–917

Gagg GM, Newman DJ, Snader KM (1997) Natural products in drug discovery and development. J Nat Prod 60:52–60

De Cal A, Pascual S, Melgarejo P (1997) Involvement of resistance induction by *Penicillium oxalicum* in the biocontrol of tomato wilt. Plant Pathol 46:72–79

De Cal A, García L.R., Pascual S, Melgarejo P (1999) Effects of timing and method of application of *Penicillium oxalicum* on efficacy and duration of control of *Fusarium* wilt of tomato. Plant Pathol 48:260–268

De Cal A, Garcia LR, Melgarejio P (2000) Induced resistance by *Penicillium oxalicum* against *Fusarium oxysporum* f. sp. lycopersici: histological studies of infected and induced tomato stems. Phytopathology 90:260–268

Gintis BO, Benson DM (1987) Biological control of *Phytophthora* root rot of azalea with *Penicillium oxalicum*. Phytopathology 77:1688

He G, Matsuura H, Takushi T, Kawano S, Yoshihara T (2004) A new antifungal metabolite from *Penicillium expansum*. J Nat Prod 67:1084–1087

Hegde VR, Silver J, Patel M, Gullo VP, Yarborough R, Huang E, Das PR, Puar MS, DiDomenico BJ, Loebenberg D (2001) Novel fungal metabolites as cell wall active antifungals: fermentation, isolation, physico-chemical properties, structure and biological activity. J Antibiot 54:74–83

Jackson M, Karwowski JP, Humphrey PE, Kohl WL, Barlow GJ, Tanaka SK (1993) Calbistrins, novel antifungal agents produced by *Penicillium restrictum*. I. Production, taxonomy of the producing organism and biological activity. J Antibiot 46:34–38

Jarvis WR, Barrie SD, Traquair JA, Stoessl A (1990) Morphological and chemical studies of *Penicillium oxalicum*, newly identified as a pathogen on greenhouse cucumbers. Can J Bot 68:21–25

Klimek-Ochab M, Lejczak B, Forlani G (2003) A metal-independent hydrolase from a *Penicillium oxalicum* strain able to use phosphonoacetic acid as the only phosphorus source. FEMS Microbiol Lett 222:205–209

Kaiserer L, Oberparleiter C, Weiler-Görz R, Burgstaller W, Leiter E, Marx F (2003) Characterization of the *Penicillium chrysogenum* antifungal protein PAF. Arch Microbiol 180:204–210

Kumagai H, Nishida H, Imamura N, Tomoda H, Omura S (1990) The structures of atpenins A4, A5 and B, new antifungal antibiotics produced by *Penicillium* sp. J Antibiot 43:1553–1558

LaMondia JA, Douglas SM (1997) Sensitivity of *Botrytis cinerea* from connecticut greenhouses to benzimidazole and dicarboximide fungicides. Plant Dis 81:729–732

Larena I, Sabuquillo P, Melgarejo P, De Cal A (2003) Biocontrol of fusarium and verticillium wilt of tomato by *Penicillium oxalicum* under greenhouse and field conditions. J Phytopathol 151:507–512

Matsukuma S, Ohtsuka T, Kotaki H, Shirai H, Sano T, Watanabe K, Nakayama N, Itezona Y, Fujiu M, Shimma N, Yokose K, Okuda T (1992) A new series of natural antifungals that inhibit P450 lanosterol C-14 demethylase 1. Taxonomy, fermentation, isolation and structural elucidation. J Antibiot 45:151–159

McGrath MT, Shishkoff N (2003) First report of the cucurbit powdery mildew fungus (*Podosphaera xanthii*) resistant to strobilurin fungicides in the United States. Plant Dis 87:1007

Nicoletti R, De Stefano M, De Stefano S, Trincone A, Marziano F (2004) Antagonism against *Rhizoctonia solani* and fungitoxic metabolite production by some *Penicillium* isolates. Mycopathologia 158:465–474

Nose H, Seki A, Yaguchi T, Hosoya A, Sasaki T, Hoshiko S, Shomura T (2000) PF1163A and B, new antifungal antibiotics produced by *Penicillium* sp. I. Taxonomy of producing strain, fermentation, isolation and biological activities. J Antibiot 53:33–37

Okada H, Kamiya S, Shiina Y, Suwa H, Nagashima M, Nakajima S, Shimokawa H, Sugiyama E, Kondo H, Kojiri K, Suda H (1998) BE-31405, a new antifungal antibiotic produced by *Penicillium minioluteum*. I. Description of producing organism, fermentation, isolation, physico-chemical and biological properties. J Antibiot 51:1081–1086

Peng X, Zhang H, Bai Z, Li B (2004) Induced resistance to *Cladosporium cucumerinum* in cucumber by pectinases extracted from *Penicillium oxalicum*. Phytoparasitica 32:377–387

Peterson SW (2000) Phylogenetic analysis of *Penicillium* species based on ITS and LSU-rDNA nucleotide sequences. In: Samson RA, Pitt JI (eds) Integration of modern taxonomic methods for *Penicillium* and *Aspergillus* classification. Harwood Academic Publishers, Amsterdam, pp 163–178, ISBN 9058231593

Phoebe CH. Jr, Combie J, Albert FG, Van Tran K, Cabrera J, Correira HJ, Guo Y, Lindermuth J, Rauert N, Galbraith W, Selitrennikoff CP (2001) Extremophilic organisms as an unexplored source for antifungal compounds. J Antibiot 54:56–65

Reddy CS, Reddy RV, Hayes AW (1981) High-performance liquid chromatographic analysis of the mycotoxin secalonic acid D and its application to biological fluids. J Chromatogr 208:17–26

Sabuquillo P, De Cal A, Melgarejo P (2005) Dispersal improvement of a powder formulation of *Penicillium oxalicum*, a biocontrol agent of tomato wilt. Plant Dis 89:1317–1323

Sallato BV, Latorre BA (2006) First report of practical resistance to QoI fungicides in *Venturia inaequalis* (Apple Scab) in Chile. Plant Dis 90:375

Sambrook J, Russell DW (2000) Molecular cloning: a laboratory manual, 3rd edn (3 Volume Set). Cold Spring Harbor Labs, Cold Spring Harbor, ISBN 0879695773

Sasaki T, Nose H, Hosoya A, Yoshida S, Kawaguchi M, Watanabe T, Usui T, Ohtsuka Y, Shomura T, Takano S, Tatsuta K (2000) PF1163 A and B, new antifungal antibiotics produced by *Penicillium* sp. II. Physico-chemical properties and structure elucidation. J Antibiot 53:38–44

Shadomy S (1987) Preclinical evaluation of antifungal agents. In: Fromtling RA (ed) Recent trends in the discovery, development and evaluation of antifungal agents. Prous Science, New Jersey, pp 8–14

Shi ZQ, Zhou MG, Ye ZY, Shi JR, Chen HG, Wang YZ (2000) Resistance monitoring of *Sclerotinia sclerotiorum* to carbendazim. Jiangsu J Agric Sci 16:226–229

Shin W, Ryu J, Choi S, Kim C, Gadagi R, Madhaiyan M, Seshadri S, Chung J, Sa T (2005) Solubilization of hardly soluble phosphates and growth promotion of maize (Zea mays L.) by *Penicillium oxalicum* isolated from rhizosphere. J Microbiol Biotechnol 15:1273–1279

Strange RN, Scott PR (2005) Plant disease: a threat to global food security. Annu Rev Phytopathol 43:83–116

Trapero-Casas A, Kaiser WJ, Ingram DM (1990) Control of *Pythium* seed rot and preemergence damping-off of chickpea in the US Pacific Northwest and Spain. Plant Dis 74:563–569

Von Dohren H, Grafe U (1997) General aspects of secondary metabolism. In: Rehm HJ, Reed G (eds) Biotechnology: products of secondary metabolism, vol 7, 2nd edn. VCH–Wiley, Weinheim, pp 2–55

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sniksy JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322, ISBN 0123721806

Yuan SK, Zhou MG (2004) Review of genetic study on fungicide resistance in phytopathogens. Acta Phytopathologica Sinica 34:3–9