# Two New *Penicillium* Species *Penicillium Dongtaiense* Sp. Nov and *Penicillium Yanchengense* sp. nov., Isolated From a Poplar Plantation in China

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# Abstract

Studies on the degradation of plant cell wall polysaccharides by fungal extracellular enzymes have attracted much recent attention. In this study, dozens of fungus species spanning genera were isolated from rotting leaves based on their ability to decompose xylan. Using genetic sequencing (rDNA internal transcribed spacer), strains were identified as members of the genera *Aspergillus*, *Penicillium*, *Alternaria*, *Campylocarpon*, *Pyrenochaeta* and *Cladosporium*. Among these strains, two *Penicillium* strains can't be assigned to any reported species. In this study, they are described new species as *Penicillium yanchengium* sp. nov$^T$ (AF 2021051) and *Penicillium dongtaiense* sp. nov$^T$ (AF 2121001) based on multigene phylogenetic analysis and morphology. *Penicillium yanchengense* sp. nov$^T$ belong to *Penicillium* section *Lanata-Divaricata* and are phylogenetically closely related to *Penicillium oxalicum* and *Penicillium asturianum*. Isolates of *Penicillium yanchengense* sp. nov$^T$ have a faster growth on Czapek yeast agar (CYA) at 37 °C, abundant exudate present on CYA, and a greater ability to produce acid on creatine sucrose agar (CREA). *Penicillium dongtaiense* sp. nov$^T$ was placed in section *Sclerotiora* and it is most closely related to *Penicillium exsudans*, *Penicillium mallochii* and *Penicillium acidum*. It is unique in slower growth on CYA and MEA plates, abundant exudate on MEA, and cerebriform grooves on YES compared to its relatives. In this study, we provide detailed description about two species.


# Introduction

Microorganisms play vital roles in various ecosystem process due to their ability to synthesize and secrete extracellular enzymes (Luo et al. 2017). These enzymes are used to decompose organic matter in the environment so as to meet the energy and nutrient requirement of microbial growth. Meanwhile, they are also involved in nutrient cycling in ecosystems including carbon, nitrogen and phosphorus cycles. In forest ecosystems, decomposition of leaf litter is an important ecological process in nutrient cycling, in which a large proportion of organic carbon is further degraded and returned to forest soil (Maggs and John 1985). As a member of decomposers, filamentous fungi play a critical role in decomposition of leaf litter because of the ability of fungal hyphae to penetrate the substrates and secrete different extracellular enzymes to break down plant cell biomass (Osono and Takeda 2002; Wardle et al. 2004).

Hemicellulose, the second most abundant renewable biomass in nature, is one of the main constituents in leaf litter and acted as the linkage between lignin and cellulose, which increases the hardness of plant cell walls (Kumar et al. 2008; Spiridon and Popa 2008). Therefore, the effective decomposition of hemicellulose is critical to the conversion of lignocellulose into high value products. Unlike cellulose, hemicelluloses are composed of heterogeneous polysaccharides including pentoses (D-xylose and D-arabinose), hexoses (D-mannose, D-glucose, and D-galactose) and uronic acids (Kumar et al. 2008; Benallaoua 2020). Thus, various enzymes are required to degrade hemicellulose completely (Benallaoua 2020). Xylan, as one of the main sugar units, accounts for 25-35 percentage in hemicellulose (Spiridon and Popa 2008). Thus, xylanase also become the most vital member in enzyme system about decomposing hemicellulose. It also has attracted increasing attention because of its wide array of

applications in fields as divergent as baking, pulp bleaching, feed industry among others (Del-Cid et al. 2013). However, the high cost of enzyme production limited their wide application. Thus, researchers have been working on a low-cost way to increase yield (Ho 2016).

Currently, industrial-scale production of xylanase mainly depends on microorganisms, such as *Trichoderma* and *Aspergillus* spp. (Li et al. 2009; da Cruz Kerber et al. 2021; Ravindran et al. 2019). Some studies have shown that fungi *Penicillium* are also excellent xylanase producers (Silva et al. 2020; Ogunyewo et al. 2020; Sunkar et al. 2020).

*Penicillium* is a well-known genus in nature that plays different roles in natural ecosystem, food industry, agriculture and medical industry. Its greatest contribution for human is production of penicillin, which revolutionized medical treatment for bacterial infections (Visagie et al. 2014a). *Penicillium* is among the most common fungi and has a worldwide distribution across habitats including soil, air, food, and plants (O'Callahan et al. 2020). *Penicillium* spp. contribute significantly to human life; some species are act as catabolic enzyme factories (Li et al. 2007; Visagie et al. 2014b; Mansouri et al. 2013) while others contribute to fermentation in the food industry, protecting dry-cured meat products from spoilage or helping improve the quality of cheese (Anelli et al. 2018; Perrone et al. 2015). Nevertheless, *Penicillium* spp. may have negative human effects. They contribute to organic material decomposition, causing food rot, or produce a variety of mycotoxins that can reduce cash crop productivity(Peterson et al. 2005).

There is a long history of using an infrageneric classification system to classify *Penicillium* and *Aspergillus*. The genus *Penicillium* belongs to the family *Aspergillaceae.* Using a polyphasic approach, combining phylogenetic analysis, phenotype and secondary metabolite production data, *Penicillium* can be subdivided in two subgenera, 32 sections, and 89 series. Currently, 483 species have been accepted (Visagie et al. 2014b; Houbraken et al. 2020). Identification of *Penicillium* species based on traditional methods is challenging due to lack of information on known species (Paul et al. 2014). However, Pitt (Pitt and Hocking 2009) has concluded that *Penicillium* can be identified at the species level according to phenotypic differences of strains grown on standardized media. Recently, phylogenetic analysis based on multi-gene sequences has been used widely to identify *Penicillium* species (Serra et al. 2008). Phylogenetic analysis of the genus *Penicillium* relies mainly on four genes: rDNA internal transcribed spacer (ITS), β-tubulin (*BenA*), calmodulin (*CaM*) and DNA-directed RNA polymerase II second largest subunit (*RPB2*). Among these, the ITS sequence is recognized as the official barcode for fungi and the *BenA* locus is accepted as the secondary identification marker for *Penicillium* (Visagie et al. 2014b; Schoch et al. 2015).

The objective of this study was to describe two new species belong to section *Sclerotiora* and *Lanata-Divaricata*, respectively. The strains were isolated during a study of screen and isolate effective hemicellulose-degrading fungi from rotting leaf.

Two isolates were identified using ITS and three other house-keeping gene sequences initially, and the result was further confirmed by morphological analysis. Detailed description about these strains are listed below.

## Materials And Methods

# Samples and Isolation

Rotting leaf litter collected from a poplar plantation located in Jiangsu Province, China was used as samples. Half gram leaf samples were cut into pieces using sterile scissors, put in a 50 mL sterilized centrifugal tube with 10 ml of sterile water, and shaken for one minute until well mixed. This mixture was subjected to a series of gradient dilutions ($10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$). An agar plate with xylan as carbon source was used as a screening medium (xylan 10 g $L^{-1}$, 4 g $L^{-1}$ $(NH_4)_2SO_4$), 1 g $L^{-1}$ peptone, 0.5 g $L^{-1}$ $MgSO_4 \cdot 7H_2O$, 1 g $L^{-1}$ $KH_2PO_4$, 20 g $L^{-1}$ agar), and adding 50 mg $L^{-1}$ streptomycin to inhibit bacterial growth. 300 µl of each diluent was coated on the media and all plates were incubated at 30 ℃ for 3–10 days in the dark. Colonies with distinctive morphological characteristics were further selected and purified on PDA medium (BD Difco, Sparks, MD, USA). All strains were stored in 20% glycerol at −80 ℃ for further analysis.

# Morphology

For morphological analysis, four standard media were used in this study (Visagie et al. 2014b): Czapek yeast autolysate agar (CYA, Kanglang, Shanghai, China), yeast extract agar (YES) (Houbraken and Samson 2011a), malt extract agar (MEA, Solarbio, Beijing, China ) and creatine agar with bromcresol purple (CREA) (FRIVSA 1985). All media were amended with trace elements (0.1 g $L^{-1}$ $ZnSO_4 \cdot 7H_2O$ and 0.05 g $L^{-1}$ $CuSO_4 \cdot 5H_2O$) for consistent conidial colors. The medium preparation and incubation conditions follow previous work (Visagie et al. 2014b). The isolated strain incubated at 25 ℃ for 7 days in the dark. Colony diameters, texture, soluble pigment, and other important characters were observed after 7 days of growth. The isolate also incubated at 5 ℃, 15 ℃, 30 ℃, and 37 ℃ on CYA for 7 days to investigate the temperature range of strain growth. An advanced inverted light microscope (IX73, Olympus, Tokyo, Japan) was used for microscopic morphology observation and image analysis was conducted with a scientific camera control (Digital Optics, Ltd., Auckland, New Zealand) equipped with OCULAR software. Colonies, cultured on MEA medium for 10 days, were used to prepare slides for microscopic observation. Uncommon hyphae was collected and dispersed in 30 µl sterile water without dye. Terms for morphological features of the *Penicillium* genus found in literature (Frisvad JC 2004) were applied to describe this fungus.

# DNA extraction, sequencing and phylogenetic analysis

Using the extracted genomic DNA from the isolate as a template, the internal transcribe spacer (ITS) was amplified using universal ITS primers (ITS4 and ITS5) (T. J. White 1990). The partial β-tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II second-largest subunit (*RPB2*) genes of three strains were PCR amplified in a GeneAmP 9700 system (Applied Biosystems, Foster City, CA, USA) using primers tub-F/tub-R (DONALDSON 1995), cal-F/cal-R(Peterson et al. 2005), and 5F2/7cR (Liu et al. 1999), respectively. The reaction condition was performed in the following sequence: Five minutes of initial denaturation at 94 ℃, followed by 35 cycles at 98 ℃ for 10 s, annealing for 30 s at 50 ℃, then elongation at 68 ℃ for 30 s,

and a final extension at 72 ℃ for 7 min (Zhu et al. 2020). PCR products were visualized using 0.8% agarose gel electrophoresis and obtained DNA sequence data were compared to available sequences in GenBank using the Blast-n program on NCBI (National Center for Biotechnology Information), and then deposited in GenBank to obtain accession numbers.

Sequences were aligned using ClustalX, then edited with BioEdit. The *RPB2, BenA, CaM* genes of the isolates and other relevant type strains were selected to conduct phylogenetic trees based on a single gene or combination of several genes. Phylogenetic analysis was performed using the neighbor-joining method with MEGA6.0. Bootstrap analysis with 1,000 iterations was conducted to determine support for each clade.

# Biochemical analysis

# Carbon source utilization

Carbon sources are necessary to maintain microbial metabolism. To investigate carbon source utilization for this strain, the carbon source identification plate FF MicroPlate™ (Biolog Inc., Hayward, CA, USA) was used to verify this biochemical property using manufacturer's instructions. The plate was placed in the dark at room temperature (25 ℃ ± 1 ℃) for 48 h. Color development of each hole was recorded.

# Enrich test

The production capacity of cyclopiazonic acid or other alkaloids of the isolate was determined by using the filter method as described by Lund (Lund 1995). Preparation of Ehrlich reagent follows the literature; 2 g 4-dimethylaminobenzaldehyde in 85 ml 96% ethanol with 15 ml 10N HCl (Frisvad JC 2004). A 4 mm diameter plug was cut from an 8 day-old colony on CYA then placed on filter paper wetted entirely with enrich reagent. Precise reaction time and color development were recorded.

## Result

# Phylogenetic analyses

The PCR-amplified sequences (*BenA*, *CaM*, and *RPB2*) of the isolates are compared and analyzed with other species available on the NCBI website using the Blast-n program (Altschul et al. 1990). The results showed that the strains were most similar to the *Penicillium* genus. Sequences were downloaded from the database of NCBI website according to the alignment results and used to construct phylogenetic trees based on single gene (*CaM*, *RPB2*) and combined gene (*BenA-CaM-RPB2*) analyses. Strains and their sequences accession numbers used in this study are listed in Table 1.

Phylogenetic position of two strains and their phylogenetic relationship with other strains were determined by analysis using neighbour-joining method. According to the analysis, *P. dongtaiense* sp. nov$^T$ (AF2021001) was clustered into section *Sclerotiora* of genus *Penicillium*. In the phylogenetic tree based on *RPB2* gene sequences, *P. dongtaiense* sp. nov$^T$ forms a clade with *Penicillium exsudans*

(HMAS248735) and *Penicillium acidum* (EML-DLW4-1). In addition, *Penicillium mallochii* (DAOM239917) appeared as sister species to this clade with a high bootstrap value of 99 (Fig. 1A). In the *CaM* NJ tree, these four strains were also clustered into a clade with a high bootstrap of 84 (Fig. 1B). The combined analysis of multiple gene sequence (*CaM*+*RPB2*+*BenA*) further indicated that the strain *P. dongtaiense* sp. nov$^{T}$ formed a monophyletic clade that also contains *P. mallochii*, *P. exsudans* and *P. acidum* (Fig. 1C). The branches of this clade are supported by bootstraps with value of 100, indicating that this phylogenetic tree is strongly resolved.

Three strains AF2021051, AF2021080 and AF2021081 were clustered into a clade without distinguish in three NJ trees. They were most closely related to the two *Penicillium* species, *P. oxalicum* (CBS219.30) and *P. asturianum* (CBS173.81) according to three phylogenetic trees. In the phylogenetic trees based on *RPB2* or *CaM* genes, the three isolates were grouped with other species belonged to section *Lanata-Divaricata* (Visagie et al. 2015a), including *P. oxalicum* (CBS219.30), *P. asturianum* (CBS173.81), *P. ludwigii* (CBS 417.68), *P. cremeogriseum* (CBS 223.66), *P. glaucoroseum* (NRRL 908), *P. janthinellum* (CBS 340.48), *P. curticaule* (CV2842), *P. limosum* (CBS 339.97), *P. brefiedianum* (NRRL710) and *P. coeruleum* (CBS 141.45). The result indicated that the isolates should be grouped into the section *Lanata-Divaricata* with certainty. When the three gene (*RPB2, BenA* and *CaM*) regions were combined to construct a phylogenetic tree, the isolates are clearly distinct from *P. oxalicum* and *P. asturianum*, indicating that these strains are a new species belonged to genus *Penicillium* based on DNA analysis.

Overall, these molecular phylogenetic analyses indicated that the isolate AF2021001 may be a new species belonged to section section *Sclerotiora* and described here as *P. dongtaiense* sp. nov$^{T}$. Other three strains AF2021051, AF2021080 and AF2021081 are belonged to section *Lanata-Divaricata* and described as *P. yanchengense* sp. nov$^{T}$. The novelty of new species was further supported by morphological observations.

# Biochemical analysis

# Carbon utilization

After incubation at 26 ℃ (± 0.5 ℃) for 2 days, color changes of each well of FF MicroPlate™ were recorded and listed in Table 2. Available carbon source to *P. dongtaiense* sp. nov$^{T}$. isolate as follows: N-Acetyl-D-glucosamine, D-Arabinose, L-Arabinose, Arbutin, D-Gluconic Acid, Glucose-1-phosphate, D-Glucuronic Acid, Glycogen, 2-Keto-D-gluconic Acid, D-Mannitol, D-Ribose, Salicin,D-Xylose, γ-Hydroxy-butyric Acid,α-Keto-glutaric Acid, D-Malic Acid, L-Malic Acid, Quinic Acid, D-Saccharic Acid, Succinic Acid, L-Alanine, L-Alanyl-glycine, L-Asparagine, L-Aspartic Acid, L-Glutamic Acid, Glycyl-L-glutamic Acid, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, Adenosine (Table 2.A). For the isolate *P. yanchengsnse* sp. nov$^{T}$, the carbon sources available are γ-Hydroxy-butyric Acid, Quinic Acid, Alaninamide, L-Alanine, L-Alanyl-glycine, L-Asparagine, L-Aspartic Acid, L-Glutamic Acid, L-Proline, L-Serine, L-Threonine (Table 2B).

# Enrich test

Production of cyclopiazonic acid or other alkaloids was identified using a filter method. The result shows that only a slightly faint yellow appears on the filter paper after 8 minutes for *P. dongtaiense* sp. nov$^{T}$ isolate (Fig. 4A). In contrast, a violet ring appeared after 10 min on the filter paper for *P. yanchengsnse* sp. nov$^{T}$ (Fig. 4B). Therefore, it can be concluded that the isolate *P. dongtaiense* sp. nov$^{T}$ produces alkaloids poorly while enrlich reaction of *P. yanchengsnse* sp. nov$^{T}$ is strong.

# Taxonomy

Penicillium dongtaiense **sp**. **nov$^{T}$**

ITS barcode: MZ298467

Alternative markers: *RPB2* = MZ327629; *BenA* = MZ313190; *CaM* =MZ476797

**Etymology:** *dongtaiense*, named after Dongtai, China where this strain was originally isolated.

Observations were recorded from colonies grown at 25 ℃ for 7 days unless stated otherwise.

Colony diameter, 7d, in mm—CYA 25 ℃, 35–38; CYA 5 ℃, 7–10; CYA 15 ℃, 24–26; CYA 30 ℃, 39–40; CYA 37 ℃, no growth; YES 25 ℃, 34–37; CREA 25 ℃, 6–10; MEA 25 ℃, 28–32.

***Colony descriptions***: On CYA 25 ℃, 7 d: colony margin on the surface but sunken at center, radially and concentrically sulcate, texture floccose, blue green at center fading to grey green until white at margin, moderate sporulation, plentiful exudate centrally present as small droplets from yellow to orange, no soluble pigments, reverse yellowish cream.

On YES 25 ℃, 7 d: colony in velvety texture, cerebriform grooves, colony margin on the surface, white, narrow, sporulation moderate, conidia dark green, centrally slight fasciculate hypha growth, no exudate, soluble pigments absent, reverse orange centrally extending to yellow.

On MEA 25 ℃, 7 d: colonies radially sulcate, plane, lightly sunk centrally, texture velutinous, floccose at margin, colored dark green to white marginal ring, moderate sporulation, prolific exudate presents as clear droplets, soluble pigments absent, reverse cream.

On CREA 25 ℃, 7 d: colonies are plane and thin, velutinous, poor acid production, reverse yellow.

Conidiophores strictly monoverticillate similar to most species in section *Sclerotiora*, stipes slightly rough walled, 200–237.5 μm×4.17–6.25 μm, ramus/branch appressed, Phialides ampulliform, 6.67–9.3 μm×28–32 μm. Conidia broadly subglobose to ellipsoidal, 10.77–11.54×12.31-16.92 μm, conidia finely smooth (Fig. 2).

**Enrich test:** No reaction.

Penicillium yanchengense **sp. nov$^{T}$**.

ITS Barcode: MW487229

Alternative markers: *BenA* = MZ327630, *RPB2* = MZ327631, *CaM* = MZ416920

**Etymology:** *yanchengense*, named after a city of Yancheng, China where the fungus was collected originally.

**Diagnosis:** A faster growth rate on CYA at 37 ℃ and on CREA at 25 ℃, relative strong production of acid on CREA. The shape of conidia is almost circular compared with other relatively close species.

**Colony diameter:** *7 days, in mm*—(descriptions based on the growth on medium at 25 ℃ unless otherwise stated) CYA 45–52; CYA 5 ℃, 5–6; CYA 15 ℃, 26–27; CYA 30 ℃, 64–65; CYA 37 ℃, 68–74; CYA 42 ℃, 7–13; MEA 36–38; YES 41–45; CREA 18–21.

## *Colony descriptions*:

On CYA at 25 ℃, 7 d: colonies nearly circular, plain, gently radially sulcate in the center, floccose in texture, colored dark green with a peripheral white band of hyphae; margin moderately wide, entire; moderate sporulation; plentiful exudates present as small clear droplets, soluble pigment absent, reverse pale yellow.

On MEA at 25 ℃, 7 d: colonies highly and centrally sulcate, relatively plane, texture floccose, mycelia dark green but white at the margin; margin moderately wide, entire; moderate sporulation, exudates droplets clear and prolific; soluble pigment absent; reverse cream.

On YES at 25 ℃, 7 d: colonies moderately deep, highly and randomly sulcate, slightly sunken at the center; colony dark green fading to blue green, margin white, narrow and entire; texture velvety, sporulation dense; no exudate, soluble pigment absent, reverse yellow.

On CREA at 25 ℃, 7 d: Moderate growth, colony plane, strong acid produced. Reverse luminous yellow.

Conidiophores mostly monoverticillate, sometimes divaricate or biverticillate, stipes slightly rough walled, 146–397×4.8–10 μm, ramus/branch appressed, 0.49–0.85 μm×2.93×3.17 1–2 branch/ramus per stipe. Phialides ampulliform, 3–6 per branch/metula, 17-24×5-7 μm (19.5±2.5×6.1±1.3 μm). Conidia broadly near-spherical and some ellipsoidal, 7.5–9×7–9.5 μm smooth walled (Fig. 3).

### Enrich test

The reaction is strong.

**Additional strains examined**: AF2021080 isolated from Yancheng, JiangSu Province. ITS barcode: OK090909 (alternative markers: *CaM* = OK127813; *BenA* = OK127816; *RPB2* = OK513275); AF2021081 isolated from Yancheng, JiangSu Province. *ITS* barcode: OK090910 (alternative markers: *CaM* = OK127817; *BenA* = OK127815; *RPB2* = OK127814)

# Discussion

*Penicillium* is one of the most common fungal genera occurring in a variety of environments, including soil, marine, vegetation, air and on numerous food products (Barbosa et al. 2018b; Visagie et al. 2014b; Park et al. 2019). Dierckx (Dierckx 1901) first proposed an infrageneric classification system in *Penicillium*, and the use of subgenera and sections has a long history in classification of *Penicillium*. In the past, most of infrageneric classifications mainly based on morphological feature such as conidiophore branching patterns, growth rates in culture. Sometimes physiological data and/or extrolite profiles could be used as further supporting evidence (Houbraken et al. 2016; Stolk and Samson 1986). Later, Houbraken and Samson (Houbraken and Samson 2011a) have reclassified *Penicilium* into 25 sections based on a multi-gene phylogeny.

DNA sequencing is a powerful taxonomic method in microbiology. The internal transcribed spacer rDNA area (ITS) is regarded as the official barcode for molecular identification of fungi. Although it works relatively well in assigning strains into one of sections, it isn't sufficiently conclusive to distinguish all closely related species in *Penicillium* (Visagie et al. 2014b). Due to its limitations in the identification of *Penicillium* sp., *BenA* was proposed as a secondary identification marker for *Penicillium*. In this study, three genes (*CaM, RPB2*, and *BenA*) were used to conduct phylogenetic analyses, and the results provide strong evidence in support of the proposed new species.

The phylogeny results indicate that the three isolates AF2021051, AF2021080 and AF2021081 belonged to section *Lanata-Divaricata* and have higher similarity with *P. oxalicum* and *P. asturianum*. Species in section *Lanata-Divaricata* mainly occur in soil, but can also be found in leaf litter or other various substrates (Barbosa et al. 2018a; Houbraken and Samson 2011b). Most of members in this section grow rapidly and present broadly spreading colonies on agar medium. The conidiophore branching patterns of these species are strongly divaricate, and they have metulae that are born terminally, subterminally and in intercalary positions (Houbraken and Samson 2011b). Generally, the growth rate and phenotypic character on CYA at 37 ℃ and acid production on CREA are most useful traits for species identification in this section (Barbosa et al. 2018a; Visagie et al. 2015b). In this study, the isolates have distinct morphological characteristics from *P. oxalicum* including a faster growth rate on CYA at 37 ℃, moderate acid production on CREA (which is poor for *P. oxalicum*) and abundant observed exudate on CYA and MEA. Exudate is absent for *P. oxalicum*. In addition, the shape of conidia of the new strain is more subround than *P. oxalicum* (Alena et al. 2018; Visagie et al. 2015a). These phenotypic characters strongly supported the novelty of the obtained strain. Additionally, the phylogenetic analyses also provide conclusive evidence for its taxonomic position in section *Lanata-Divaricata*. Although the isolates are closely related to *P. oxalicum* and *P. asturianum*, they are significantly different from these two species in both single gene and combined phylogenetic trees.

The strain AF2021001 belonged to section *Sclerotiora* based on phylogenetic analysis. The *RPB2*- and *CaM*-based phylogram demonstrated the close relationship between the strain AF2021001 and its sister species *P. exsudans*, *P. mallochii* and *P. acidum*. The NJ phylogenetic analysis of a combined alignment

of *CaM*, *RPB2* and *BenA* gene sequences showed a distinctive separation between isolate AF2021001 and its closely species with a high bootstrap values of 100. Therefore, phylogenetic analysis provides powerful evidence to access the taxonomic position of the new proposed strain AF2021001 in section *Sclerotiora*, genus *Penicillium*.

Members of section *Sclerotiora* are characterized by production of bright yellow or orange pigment in their mycelia, sclerotia, ascocarps. They produce soluble pigments or colony reverse and the majority of species produce monoverticillate conidiophores; with the exception of *P. herquei*, *P. malachiteum and P. nodositatum* (Houbraken and Samson 2011a; Rivera and Seifert 2011).We compared the phenotypic characters of AF2021001strain and its close relatives to further explore its novelty. In their macro-morphology, strain AF2021001 showed slightly more growth on CYA at 5 ℃. However, its sister species did not grow under the same conditions except *P. acidum*, which was significantly different from *P. exsudans* and *P. mallochii*. The growth rate of this isolate on MEA and YES at 25℃ is good but slightly slower than *P. exsudans* and *P. mallochii*. It has rapidly growth on YES and CYA at 25 ℃ compared to *P. acidum* (Wanasinghe et al. 2018). Colonies of isolate AF2021001 on YES have cerebriform grooves while *P. exsudans* is radially sulcate. Another notable character of strain AF2021001 is the presence of a large amount of exudate as clear droplets on MEA where it is absent in *P. exsudans* and *P. mallochii* (Wang et al. 2017; Rivera et al. 2012). Additionally, it produces poor acid compared to *P. acidum* on CREA (Wanasinghe et al. 2018).

These findings strongly suggest that these strains isolated from leaf litter are two new species belonging to genus *Penicillium* with great potential for hemicellulase production.

# Declarations

### Authors and contributors

S.H and P.H contributed equally to this work. S.H and P.H performed experiments and wrote the original manuscript. P.H and F.-J.J designed the experiments. S.H, X.-Y.Y and B.-T.W. analyzed the data and conducted reference search. P.H and F.-J.J. reviewed and edited the manuscript. All authors have read and agreed to the submitted version of the manuscript.

### Data availability statement

Sequence data generated from the four isolates in this study have been submitted and deposited in GenBank. All strains are deposited in China Center for Typical Culture Collection (CCTCC). The type strain ***Penicillium yanchengense*** **sp. nov$^T$** was deposited with the accession number AF 2021051. The accession numbers of sequence are MW487229 (ITS), MZ327630 (*BenA*), MZ416920 (*CaM*) and MZ32763 (*RPB2*). Additional strains examined: AF2021080 isolated from Yancheng, JiangSu Province. ITS barcode: OK090909 (alternative markers: *CaM* = OK127813; *BenA* = OK127816; *RPB2* = OK513275); AF2021081 isolated from Yancheng, JiangSu Province. ITS barcode: OK090910 (alternative markers: *CaM* = OK127817; *BenA* = OK127815; *RPB2* = OK127814). The other strain ***Penicillium dongtaiense*** **sp.**

**nov**$^{T}$ was also deposited with the accession number AF 2021001. The accession numbers of sequence are ITS region sequence, MZ298467; *BenA* region sequence, MZ313190; *RPB2* region sequence, MZ327629; *CaM* region sequence, MZ476797.

### Conflicts of interest

The authors declare that there are no conflicts of interest.


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# Tables

**Table1** *Penicillium* strains and Genebank accession number used in this study.

| Species | strains | Genebank accession number | | |
|---|---|---|---|---|
| | | *BenA* | *RPB2* | *CaM* |
| ***P. yanchengense* sp. nov$^T$** | AF2021051 | MZ327630 | MZ327631 | MZ416920 |
| ***P. yanchengense* sp. nov** | AF2021080 | OK127816 | OK513275 | OK127813 |
| ***P. yanchengense* sp. nov** | AF2021081 | OK127815 | OK127814 | OK127817 |
| ***P. dongtaiense* sp. nov$^T$** | AF2021001 | MZ313190 | MZ327629 | MZ476797 |
| *P. oxalicum* | CBS 219.30 | KF296462.1 | JN121456.1 | MN969283.1 |
| *P. reticulisporum* | CBS 122.68 | MN969394.1 | KF296454.1 | MN969293.1 |
| *P. elleniae* | CBS 118135 | - | KF296429.1 | MN969254.1 |
| *P. limosum* | CBS 339.97 | GU981621.1 | KF296433.1 | MN969271.1 |
| *P. luzoniacum* | CBS 622.72 | - | JN406544.1 | KP016790.1 |
| *P. dimorphosporum* | NRRL 5207 | - | KF900187.1 | - |
| *P. canis* | NRRL 62798 | - | KF900196.1 | - |
| *P. ludwigii* | CBS 417.68 | KF296468.1 | KF296435.1 | MN969273.1 |
| *P. cremeogriseum* | CBS 223.66 | DQ834936.1 | KF296426.1 | MN969250.1 |
| *P. mbrefeldianum* | NRRL 710 | EU021669.1 | EU021658.1 | EU021683.1 |
| *P. janthinellum* | CBS 340.48 | GU981625.1 | JN121497.1 | MN969268.1 |
| *P. coeruleum* | CBS 141.45 | - | KF296425.1 | MN969247.1 |
| *P. glaucoroseum* | NRRL908 | KF296469.1 | KF296430.1 | KF296400.1 |
| *P. curticaule* | CV2842 | JX091526.1 | KF296417.1 | JX141536.1 |
| *P. asturianum* | CBS 173.81 | KF296470.1 | KF296416.1 | KF296366.1 |
| *P. subturcoseum* | CV2835=CBS:139132 | - | - | JX157532.1 |
| *P. cravenianum* | CV0092=CBS 139138=DAOM 241082=DTO 180-I5 | - | - | JX157418.1 |
| *P. levitum* | NRRL 705=ATCC 10464=CBS 345.48=IMI 039735 | - | - | JN714939.1 |

| | | | | |
|---|---|---|---|---|
| *P. exsudans* | HMAS248735 | KX885042.1 | KX885033.1 | KX885052.1 |
| *P. mallochii* | DAOM239917 | JN625973.1 | KX961296.1 | JN626016.1 |
| *P. sclerotiorum* | CBS287.36=NRRL2074 | - | JN406585.1 | JN626044.1 |
| *P. austrosinicum* | HMAS248734 | KX885041.1 | KX885032.1 | KX885051.1 |
| *P. circulare* | CNUFC-GEU220-1 | MK481057.1 | MK481053.1 | MK481061.1 |
| *P. guanacastense* | DAOM239912 | JN625967.1 | KX961295.1 | JN626010.1 |
| *P. hirayamae* | CBS229.60 | JN625955.1 | JN121459.1 | FJ530978.1 |
| *P. maximae* | CBS134565 | - | MN969126.1 | KC773821.1 |
| *P. choerospondiatis* | HMAS248813 | KX885043.1 | KX885034.1 | KX885053.1 |
| *P. jugoslavicum* | CBS192.87 | KC773789.1 | JN406618.1 | KC773815.1 |
| *P. brocae* | CBS116113=NRRL31472 | KC773787.1 | JN406639.1 | AY741737.1 |
| *P. verrucisporum* | HMAS248819 | KX885049.1 | KX885040.1 | KX885059.1 |
| *P. alexiae* | CBS134558 | - | KX961291.1 | - |
| *P. adametzioides* | CBS313.59 | JN799642.1 | JN406578.1 | JN686387.1 |
| *P. herquei* | CBS336.48 | - | JN121494.1 | - |
| *P. acidum* | EML-DLW4-1 | KY587439.1 | KY587446.1 | KY587442.1 |
| *P. vanoranjei* | DTO99H6 | - | - | KC695691.1 |
| *P. fusisporum* | AS3.15338 | - | - | KF769413.1 |
| *P. mellis* | CBS 142499 | - | - | MN969327.1 |
| *P. johnkrugii* | DAOM 239943 | - | - | JN686401.1 |
| *P. jacksonii* | DAOM 239937 | - | - | JN686391.1 |
| *P. cainii* | DAOM 239914 | - | - | JN686389.1 |
| *P. luzoniacum* | CBS622.72 | KP016759.1 | JN406544.1 | KP016790.1 |
| *P. canis* | NRRL62798 | KF900167.1 | KF900196.1 | KF900177.1 |
| *Aspergillus aflatoxiformans* | DTO 228-G2 | MG517706.1 | MG517897.1 | MG518076.1 |

**Table 2** Carbon source utilization of two strains.

Growth reaction: no reaction −; poor reaction +; moderate reaction ++ ; strong reaction +++.

**A:** Carbon source utilization of *Penicillium dongtaiense* sp. nov$^{T}$ (AF2021001).

| Carbon source | reaction | Carbon source | reaction | Carbon source | reaction |
|---|---|---|---|---|---|
| Water | − | Lactulose | − | γ-Hydroxy-butyric Acid | +++ |
| Tween 80 | + | Maltitol | − | p-Hydroxyphenylacetic Acid | ++ |
| N-Acetyl-D-galactosamine | − | Maltose | − | α-Keto-glutaric Acid | +++ |
| N-Acetyl-D-glucosamine | +++ | Maltotriose | − | D-Lactic Acid Methyl Ester | + |
| N-Acetyl-D-mannosamine | − | D-Mannitol | +++ | L-Lactic Acid | + |
| Adonitol | + | D-Mannose | + | D-Malic Acid | +++ |
| Amygdalin | − | D-Melezitose | − | L-Malic Acid | +++ |
| D-Arabinose | +++ | D-Melibiose | + | Quinic Acid | +++ |
| L-Arabinose | +++ | α-Methyl-D-galactoside | − | D-Saccharic Acid | +++ |
| D-Arabitol | − | β-Methyl-D-galactoside | − | Sebacic Acid | + |
| Arbutin | +++ | α-Methyl-D-glucoside | − | Succinamic Acid | − |
| D-Cellobiose | − | β-Methyl-D-glucoside | + | Succinic Acid | +++ |
| α-Cyclodextrin | + | Palatinose | − | SuccinicAcidMono-mMethyl Ester | + |
| β-Cyclodextrin | + | D-Psicose | − | N-Acetyl-L-glutamic Acid | + |
| Dextrin | + | D-Raffinose | + | Alaninamide | ++ |
| i-Erythritol | − | L-Rhamnose | − | L-Alanine | +++ |
| D-Fructose | + | D-Ribose | +++ | L-Alanyl-glycine | +++ |
| L-Fucose | − | Salicin | +++ | L-Asparagine | +++ |
| D-Galactose | − | Sedoheptulosan | − | L-Aspartic Acid | +++ |
| D-Galacturonic Acid | − | D-Sorbitol | − | L-Glutamic Acid | +++ |
| Gentiobiose | + | L-Sorbose | ++ | Glycyl-L-glutamic Acid | +++ |
| D-Gluconic Acid | +++ | Stachyose | ++ | L-Ornithine | ++ |
| D-Glucosamine | − | Sucrose | + | L-Phenylalanine | +++ |

| | | | | | |
|---|---|---|---|---|---|
| α-D-Glucose | + | D-Tagatose | − | L-Proline | +++ |
| Glucose-1-phosphate | +++ | D-Trehalose | − | L-Pyroglutamic Acid | − |
| Glucuronamide | − | Turanose | − | L-Serine | +++ |
| D-Glucuronic Acid | +++ | Xylitol | + | L-Threonine | +++ |
| Glycerol | ++ | D-Xylose | +++ | 2-Amino Ethanol | − |
| Glycogen | +++ | γ-Amino-butyric Acid | − | Putrescine | ++ |
| m-Inositol | + | Bromosuccinic Acid | − | Adenosine | +++ |
| 2-Keto-D-gluconic Acid | +++ | Fumaric Acid | + | Uridine | ++ |
| α-D-Lactose | − | β-Hydroxy-butyric Acid | ++ | Adenosine-5'-Monophosphate | ++ |
| | | | | | |

**B**: Carbon source of *Penicillium yanchengense* sp. nov$^{T}$

| Carbon source | reaction | Carbon source | reaction | Carbon source | reaction |
|---|---|---|---|---|---|
| Water | | Lactulose | + | γ-Hydroxy-butyric Acid | +++ |
| Tween 80 | + | Maltitol | + | p-Hydroxyphenylacetic Acid | - |
| N-Acetyl-D-galactosamine | - | Maltose | ++ | α-Keto-glutaric Acid | + |
| N-Acetyl-D-glucosamine | ++ | Maltotriose | ++ | D-Lactic Acid Methyl Ester | - |
| N-Acetyl-D-mannosamine | - | D-Mannitol | + | L-Lactic Acid | + |
| Adonitol | + | D-Mannose | ++ | D-Malic Acid | ++ |
| Amygdalin | + | D-Melezitose | + | L-Malic Acid | ++ |
| D-Arabinose | ++ | D-Melibiose | + | Quinic Acid | +++ |
| L-Arabinose | ++ | α-Methyl-D-galactoside | - | D-Saccharic Acid | ++ |
| D-Arabitol | ++ | β-Methyl-D-galactoside | + | Sebacic Acid | - |
| Arbutin | + | α-Methyl-D-glucoside | - | Succinamic Acid | + |
| D-Cellobiose | ++ | β-Methyl-D-glucoside | + | Succinic Acid | ++ |
| α-Cyclodextrin | - | Palatinose | + | SuccinicAcidMono-mMethyl Ester | + |
| β-Cyclodextrin | + | D-Psicose | + | N-Acetyl-L-glutamic Acid | - |
| Dextrin | + | D-Raffinose | + | Alaninamide | +++ |
| i-Erythritol | ++ | L-Rhamnose | + | L-Alanine | +++ |
| D-Fructose | ++ | D-Ribose | ++ | L-Alanyl-glycine | +++ |
| L-Fucose | - | Salicin | + | L-Asparagine | +++ |
| D-Galactose | + | Sedoheptulosan | - | L-Aspartic Acid | +++ |
| D-Galacturonic Acid | + | D-Sorbitol | + | L-Glutamic Acid | +++ |
| Gentiobiose | + | L-Sorbose | - | Glycyl-L-glutamic Acid | ++ |
| D-Gluconic Acid | ++ | Stachyose | + | L-Ornithine | ++ |
| D-Glucosamine | - | Sucrose | + | L-Phenylalanine | ++ |

| | | | | | |
|---|---|---|---|---|---|
| α-D-Glucose | ++ | D-Tagatose | - | L-Proline | +++ |
| Glucose-1-phosphate | ++ | D-Trehalose | + | L-Pyroglutamic Acid | - |
| Glucuronamide | - | Turanose | ++ | L-Serine | +++ |
| D-Glucuronic Acid | ++ | Xylitol | + | L-Threonine | +++ |
| Glycerol | ++ | D-Xylose | ++ | 2-Amino Ethanol | + |
| Glycogen | ++ | γ-Amino-butyric Acid | ++ | Putrescine | + |
| m-Inositol | - | Bromosuccinic Acid | ++ | Adenosine | + |
| 2-Keto-D-gluconic Acid | ++ | Fumaric Acid | + | Uridine | + |
| α-D-Lactose | + | β-Hydroxy-butyric Acid | ++ | Adenosine-5'-Monophosphate | + |

# Figures

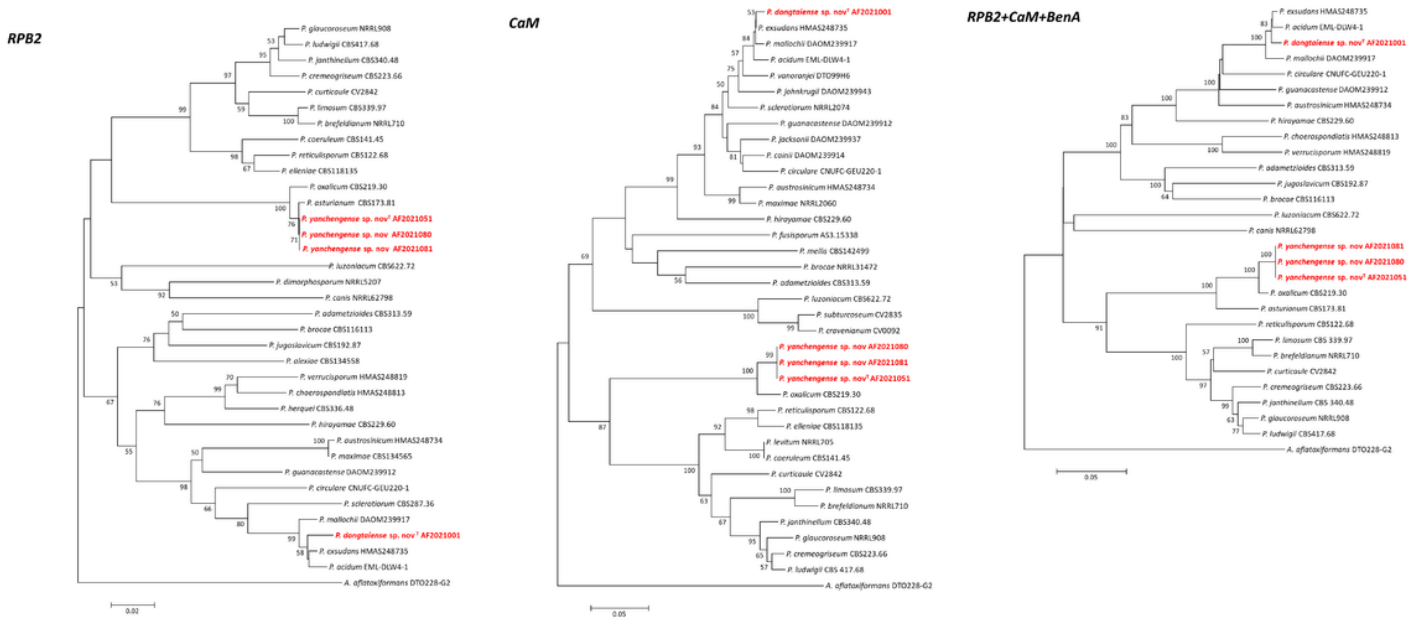
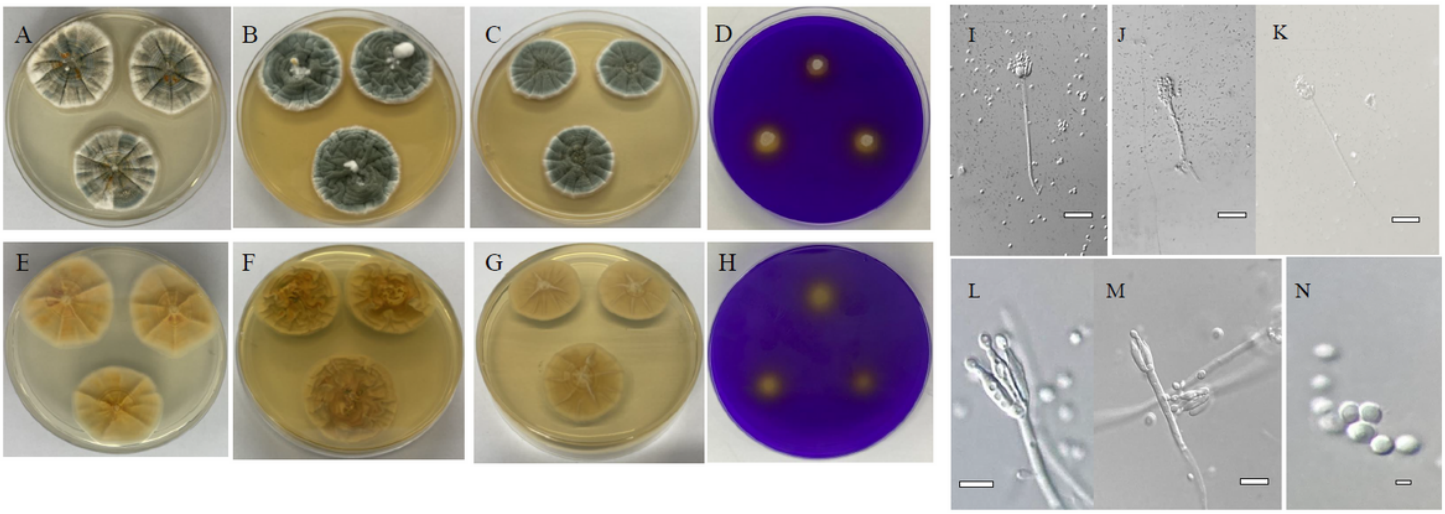



**Figure 1**

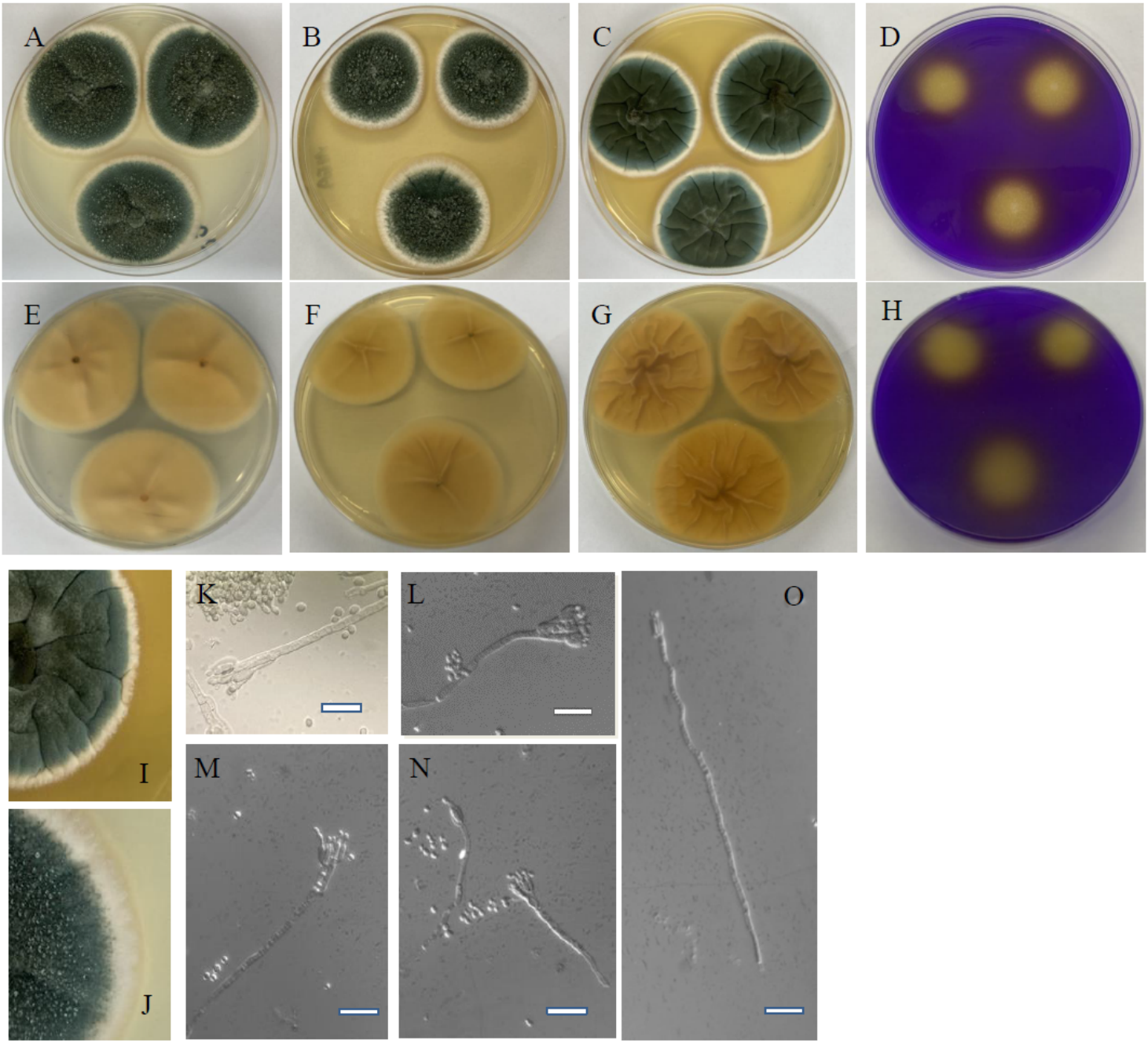
A. Phylogenetic tree generated from neighbor-joining analysis based on the single RPB2 region. Numbers at nodes represent bootstraps values (1000 replications). Only bootstrap values >50% are shown. Bars: 0.02 expected nucleotide substitutions per site. Aspergillus aflatoxiformans DTO228-G2 is chosen as an outgroup. B. Phylogenetic tree based on CaM sequence data using neighbour-joining analysis. Numbers at nodes represent bootstraps values (1000 replications). Only bootstrap values >50% are shown. Bars:

0.05 expected nucleotide substitutions per site. Aspergillus aflatoxiformans DTO228-G2 is chosen as an outgroup. C. Phylogenetic tree based on a combination of CaM, RPB2 and BenA sequence data. Numbers at nodes represent bootstraps values (1000 replications). Only bootstrap values >50% are shown. Bars, 0.05 expected nucleotide substitutions per site. Aspergillus. Aflatoxiformans DTO228-G2 is chosen as an outgroup.




## Figure 2

Penicillium dongtaiense sp. novT (AF2021001). All cultures shown after 7 d growth at 25 °C.A, B, C, D colonies on CYA, YES, MEA, CREA (all obverse); E, F, G, H colonies on CYA, YES, MEA, CREA (all reverse). I-N Conidiophores. Scale bars I-K=50μm, L-M=20μm, N=10μm.




## Figure 3

Penicillium yanchengense sp. novT (AF2021051). Cultures shown after 7 days of growth on four media at 25 °C. A: CYA□B: MEA, C: YES, D: CREA, E: CYA reverse F: MEA reverse, G: YES reverse, H: CREA reverse. I: texture on YES; J: texture on CYA. K-O conidiophores. Scale bars K-O=50 µm.

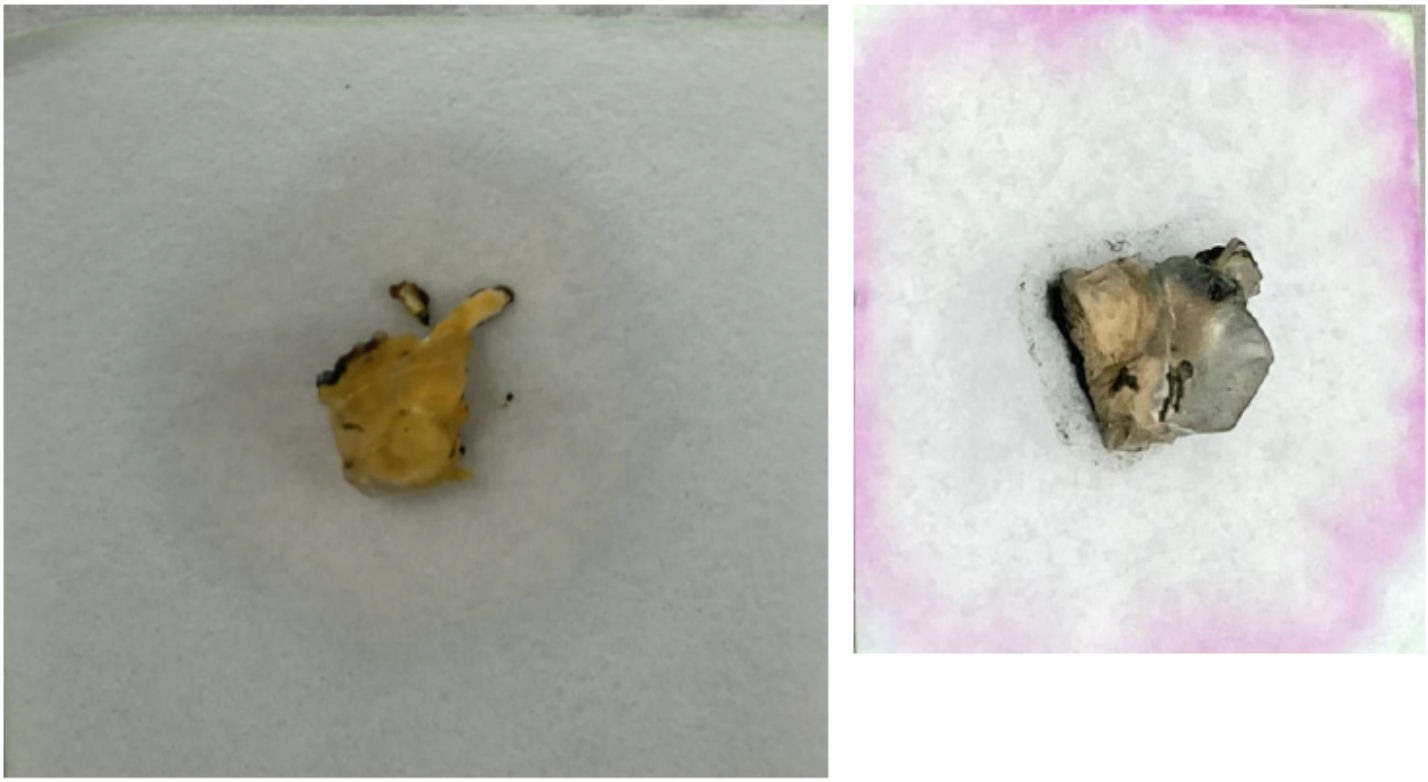


**Figure 4**

Enrich test. The isolated strains incubated on the CYA medium at 25 °C for 8 days. A: Violet in Penicillium dongtaiense sp. novT (AF2021001). B: Violet in Penicillium yanchengense sp. novT (AF2021051).