

## One new record of *Penicillium* isolated from central air-conditioning system of hospitals in China

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**Abstract:** [Objective] The objective of this study was to examine taxonomic position of one *Penicillium* strains isolated from central air-conditioning systems of hospitals in China. [Methods] *Penicillium* spores were collected on Sabourand's agar by the 6-stage Andersen impactor. Then one strain was indentified by analysis of  $\beta$ -tubulin gene and morphological characters. For phylogenetic analyses in MEGA 4.0,  $\beta$ -tubulin gene sequences were analyzed using the maximum parsimony approach of close-neighbor-interchange algorithm, in which the initial trees were obtained with the random addition of sequences (1 000 replicates). [Results] In this study, we made a survey about fungi group in central air-conditioning systems in several hospitals in China. One new record *Penicillium bilaiae* SW11-6 was isolated from the central air-conditioning system in one hospital in Beijing, China, which was described and illustrated. Macroscopic and microscopic morphology was observed and described. The phylogeny of this new record and related species was constructed based on  $\beta$ -tubulin sequences alignment. The frequency of SW11-6 is the second one in *Penicillium* in the detected samples. [Conclusion] Utilizing comprehensive approaches of morphological characters and molecular identification, we concluded that we found one new Chinese record species of the *Penicillium*, strain SW11-6 was identified as *P. bilaiae* Chalabuda. And whether it will do harm to human's health needs further studying.

**Keywords:** Central air-conditioning systems, Phylogeny, Taxonomy, *Penicillium bilaiae*

## 中国医院集中空调系统中的一株青霉新记录种

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**摘要:** 【目的】明确我国医院中分离得到的一株青霉的分类地位。【方法】采用六级梯度撞击式空气采样法获得医院空气样品,在SA培养基上分离得到青霉菌,通过形态学鉴定和 $\beta$ -Tubulin序列对菌株进行初步鉴定;选取MEGA 4.0软件用Bootstrap方法构建 $\beta$ -Tubulin序列系统进化树并分析其亲缘关系。【结果】对北京市的医院集中空调系统的污染真菌群落进行调查,发现

**Foundation item:** This work was supported by the Ministry of Health Public Research Foundation (No. 201002001)

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**Received:** September 05, 2013; **Accepted:** December 06, 2013; **Published online:** December 11, 2013

一株青霉新记录种, 即 *Penicillium bilaiae*。本文对其菌落和微观形态进行了详述, 并根据  $\beta$ -Tubulin 序列建立了系统发育树。*P. bilaiae* 是被检样品中青霉属第二优势菌种。【结论】结合形态学特征和分子鉴定结果, 判定菌株 SW11-6 为比莱青霉(*P. bilaiae*), 即发现青霉属一株中国新记录种, 但其是否对人类健康存在潜在危害还有待进一步研究。

关键词: 集中空调系统, 系统进化, 分类学, 比莱青霉

## 1 Introduction

Central air-conditioning systems are used widely all over the world for providing thermal comfort. The buildings using central air-conditioning systems are more air tight and poorly ventilated than the common ones. Therefore, if the central air-conditioning systems were polluted by harmful microorganism, it will lead to human health issues. According to previous reports, the dominant fungi in central air-conditioning system are *Penicillium*, *Cladosporium* and *Aspergillus*<sup>[1]</sup>. Among them *Penicillium* were reported as common fungal allergens, which could cause allergic rhinitis, allergic asthma and kinds of inflammatory conditions<sup>[2-3]</sup>.

The present study examined the fungal community composition in central air-conditioning system. During a survey of fungi in central air-conditioning system of hospitals in Beijing, China, one new records namely *Penicillium bilaiae* was isolated and identified.

## 2 Materials and Methods

### 2.1 Sampling

Sampling was conducted at central air-conditioning systems in 3 hospitals in Beijing, December 2011 (Table 1). Five rooms (or departments) in each hospital, two sites per room, were selected, respectively.

Fungi spores were collected on Sabourand's agar (SA: peptone 10 g, glucose 40 g, agar 20 g, distilled water 1 L) by the 6-stage Andersen impactor (with aerodynamic cut-size diameters of 7.00, 4.70, 3.30, 2.10, 1.10 and 0.65  $\mu\text{m}$ ) located at 50 cm from

air supply outlet, drawing air at a flow rate of 28.3 L/min, for 5 min. All Sabourand's agar plates were transported to the lab within 3 hours and incubated at 25 °C.

### 2.2 Isolation and identification

Five days after incubation, visible fungal spores were picked up from conidiophores with a sterile inoculating needle and transferred to MEA (malt extract 20 g, peptone 1.0 g, agar 20 g, distilled water 1 L) at 25 °C. Through the microscope, one strain was picked up which has the same fungal microscopic morphology with others. For morphological studies, *Penicillium* isolated were inoculated onto Czapek yeast extract agar (CYA:  $\text{K}_2\text{HPO}_4$  1.0 g, Czapek concentrate 10 mL, yeast extract 5 g, sucrose 30 g, agar 15 g, distilled water 1 L), Czapek agar ( $\text{NaNO}_3$  3.0 g,  $\text{K}_2\text{HPO}_4$  1.0 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.5 g,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.01 g, KCl 0.5 g, sucrose 30 g, agar 15 g, distilled water 1 L) and 25% glycerol nitrate agar (G25N:  $\text{K}_2\text{HPO}_4$  0.75 g, Czapek concentrate 7.5 mL, yeast extract 3.7 g, glycerol 250 g, agar 12 g, distilled water 750 mL). Five duplicates each medium. Inoculate 3 spots on each dish with sterile inoculating needle quickly. Colony appearance, exudate production, pigmentation and reverse coloration were assessed and colony diameters were measured and recorded after 7 days of incubation at 25 °C. Colony color was assessed according to The Methuen Handbook of Color by Kornerup and Wanscher<sup>[4]</sup>. For microscopic morphology, the water mounts were used for all observations, measurements and photographs under a Nikon 80i microscope with DIC<sup>[5]</sup>. The average of 50–100 spores and 50–100 conidiophores

Table 1 Microenvironment of three hospitals in survey  
表 1 所调查的 3 家医院的微环境

Place	Temperature (°C)	Humidity (%)	Velocity (m/s)	PM10 (mg/m <sup>3</sup> )	PM2.5 (mg/m <sup>3</sup> )	CO <sub>2</sub> (%)
Fengtai Hospital	22.4	27.6	0.02	0.041	0.043	0.128
Boai Hospital	24.9	45.6	0.04	0.066	0.062	0.068
307 PLA Hospital	26.4	31.2	0.35	0.023	0.043	0.070

were recorded. The arithmetic means and standard errors were calculated based on approximately 30 measurements.

### 2.3 DNA phylogeny

Fungal cultures were grown on MEA for 7 days at 25 °C. Ten to 50 mg of fresh mycelium were collected and placed in 1.5 mL eppendorf tube. DNA was extracted by Plant Genomic DNA kit (TIANGEN BIOTECH BEIJING Co., Ltd.).  $\beta$ -tubulin gene was amplified using, primers Bt2a (5'-GGTAACCAAATCGGTGCTGCTTTC-3') and Bt2b (5'-ACCCTCAGTGATGACCCCTTGGC-3')<sup>[6]</sup>. Sequences for selected strains together with reference sequences obtained from GenBank were aligned using Clustal X<sup>[7]</sup>. Alignment was manually adjusted where necessary with MEGA version 4.0<sup>[8]</sup>. Cladistics analyses using the Neighbor-Joining method was performed with the same program<sup>[9]</sup>. The Neighbor-Joining tree was constructed with Kimura 2-parameter model, including transitions and transversions and with pairwise deletion of gaps. Clade stability was assessed in a bootstrap analysis with 1 000 replicates.

### 2.4 Data statistics

The fungal strains were identified morphologically for calculating the concentrations, colony forming unit/m<sup>3</sup> (CFU/m<sup>3</sup>). Shown in following formula was used to calculate fungi concentrations:

$$\text{CFU/m}^3 = \frac{\text{CFU}_{\text{petri}} \times 1\,000}{28.3 \times 5}$$

$$\text{Frequency (100\%)} = \frac{\text{Isolates number}}{\text{Total fungi number}} \times 100.$$

## 3 Results

### 3.1 Taxonomic description

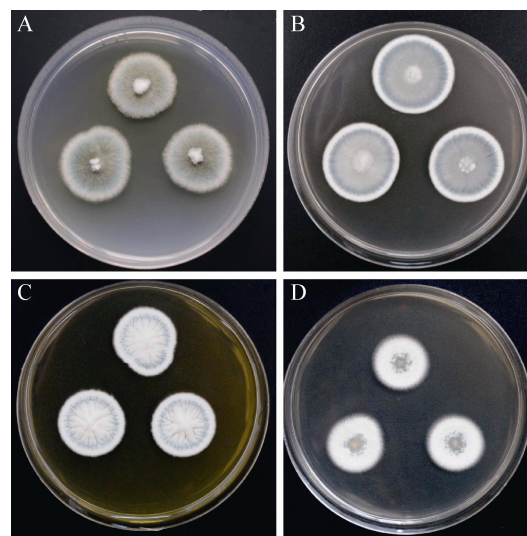
*Penicillium bilaiae* Chalabuda Not. Syst. Crypt. Inst. Bot. Acad. Sci. URSS 6: 165, 1950

Figure 1, 2

*Penicillium lilacinoechinulatum* Abe, J. Aen. Appl. Microbiol., Tokyo 2: 54, 1956. (nom. Inval., Art. 36).

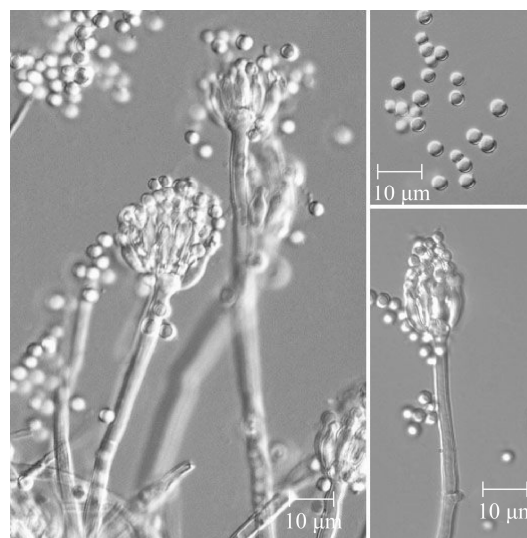
*Penicillium lilacinoechinulatum* Abe ex G. Smith, Trans. Br. Mycol. Soc. 46: 335, 1963.

On CA at 25 °C within 7 days: Colonies typically 27–28 mm in diam, plane or radially sulcate, floccose to funiculose with white mycelium;



**Figure 1** *Penicillium bilaiae*: colonies on CA (A), CYA (B), MEA (C), and G25N (D) at 25 °C, after 7 days

图1 *Penicillium bilaiae* 在 CA (A)、CYA (B)、MEA (C) 和 G25N (D) 上 25 °C 培养 7 d 后的菌落形态



**Figure 2** *Penicillium bilaiae*: Penicilli and conidia on CYA

图2 *Penicillium bilaiae* 在 CYA 上的菌丝和孢子显微形态

margins wide, usually deep; conidiogenesis light to moderate, Dull Green (M.25-26D3) or Glaucous Grey (Rayner, 109); exudates sometimes present, clear or brown; brown soluble pigment usually produced; reverse typically deep Reddish Brown (M.9E-F8) or Bay (Rayner, 6), paler in some isolates.

On CYA at 25 °C within 7 days: Colonies typically 28–30 mm in diam, plane or radially sulcate, with floccose to funiculose white mycelium; margins wide, usually deep; conidiogenesis light to moderate, Dull Green (M.25-26D3) or Glaucous Grey (Rayner, 109); exudates sometimes present, clear or brown; brown soluble pigment usually produced; reverse typically deep Reddish Brown (M.9E-F8) or Bay (Rayner, 6), paler in some isolates.

On MEA at 25 °C within 7 days: Colonies 25–26 mm in diam, plane or lightly radially sulcate, typically low to moderately deep, very deep (5 mm) with white mycelium produced by some isolates, surface texture velutinous to markedly obviously funiculose; margins low and wide; conidiogenesis light to moderate, Dull Green (M.25-27D3) or Glaucous Grey (Rayner, 109); exudates absent; brown to dark brown soluble pigment sometimes produced; reverse pale, golden brown or deep brown.

On G25N at 25 °C within 7 days: Colonies 12–15 mm in diam, plane or radially sulcate, floccose to funiculose; mycelium white or cream; conidiogenesis sparse, coloured as on CYA, or absent; exudate not produced; brown soluble pigment sometimes present; reverse pale or yellow brown depending on soluble pigment production.

On CYA at 5 °C within 7 days: No germination to germination by a proportion of conidia.

On CYA at 37 °C within 7 days: Sometimes no growth, sometimes colonies as large as 10–15 mm in diam, convolute, white, irregularly margined; conidiogenesis sparse or absent; exudates absent; brown or yellow soluble pigment usually produced; reverse yellow to brown.

Conidiophores borne from aerial hyphae, either solitary or in funicles, stipes (31–49)  $\mu\text{m} \times$  (2.2–2.5)  $\mu\text{m}$ , smooth walled, straight or sinuous, strictly monoverticillate, terminally spatulate or vesiculate up to 6  $\mu\text{m}$  in diam; phialides crowded, parallel, in verticals of up to 24, ampulliform, (6–8)  $\mu\text{m} \times$  (2.0–2.5)  $\mu\text{m}$ , with short collula; conidia subspheroidal to ellipsoidal, (2.5–3.0)  $\mu\text{m} \times$  (2.3–2.5)  $\mu\text{m}$ , with rugulose to rugose walls, borne in well defined columns distinguishing characteristics.

Habitat: Dead stems.

Distribution: UK (Yadav, 1960), China.

Material examined: Central air-conditioning

systems, from Fengtai Hospital, Beijing. CGMCC3.17105.

*Notes:* *Penicillium bilaiae* shares many characters in common with *P. chermesinum*. Like *P. chermesinum*, *P. bilaiae* produces relatively short vesiculate conidiophores from funicles of aerial hyphae<sup>[10]</sup>. However, *P. bilaiae* grows more slowly on CYA at 25 °C and 37 °C on MEA, than does *P. chermesinum*, and produces conidia which are rugulose to rugose.

### 3.2 Sequence analysis of $\beta$ -tubulin gene

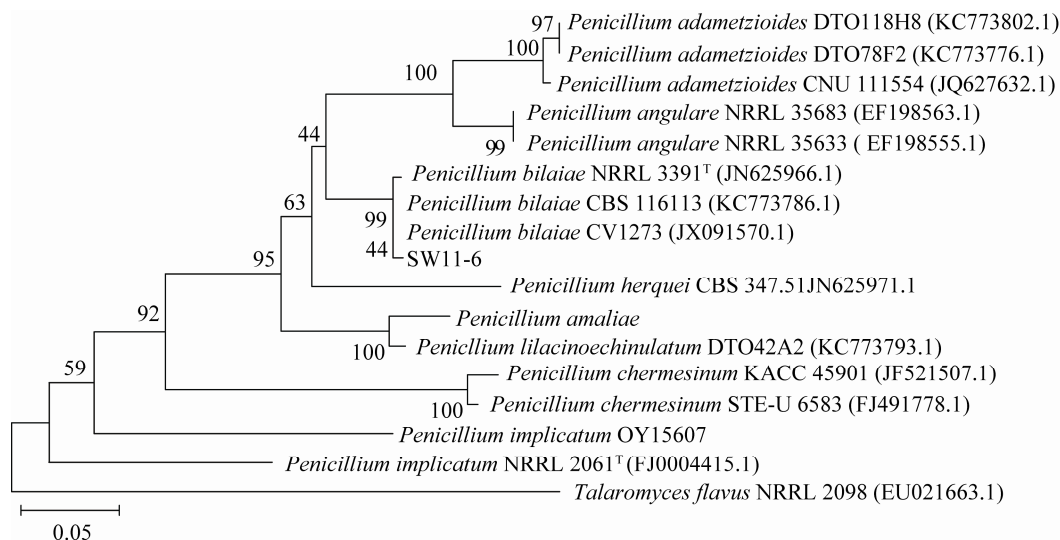
The partial  $\beta$ -tubulin gene of *Penicillium bilaiae* from air-conditioning was amplified. Amplification of the  $\beta$ -tubulin gene with primers Bt2a and Bt2b yields fragment of approximately 500 bp. The resulting sequences and relevant sequences available in the GenBank database were initially aligned with the Clustal X program. BLAST database searches were performed with partial  $\beta$ -tubulin gene as queries to reveal relationships to published sequences.  $\beta$ -tubulin gene sequences of the isolates SW11-6 isolates were identical to the type strain NRRL 3991 of *P. bilaiae* (Figure 3). Meanwhile, in the phylogenetic tree, the isolate placed in a clade comprising reference isolates of *P. bilaiae* with 100% bootstrap values support. The results indicated that the isolates SW11-6 was *P. bilaiae*.

### 3.3 Frequency of *Penicillium bilaiae* in central air-conditioning system

In Fengtai hospital, seven isolates of *Penicillium bilaiae* were detected. In hospital air samples, *Penicillium* was the most encountered genus, *P. bilaiae* was the second predominant species in isolated *Penicillium* (Table 2).

## 4 Discussion

The type strain of *Penicillium bilaiae* was isolated from soil. Cunningham J. E. et al. reported that *P. bilaiae* was a species of native soil fungus that can be used as a plant growth-promoting microorganism (PGPM)<sup>[11]</sup>. Other habitats including glasshouse, have been reported. According to our study, *P. bilaiae* was firstly reported from central air-conditioning system. The internal environment of central air-conditioning system is very special. It is drier, more dull and jejune compared to soil. Up to now, there are a few of literatures reported the



**Figure 3 Neighbor-Joining tree based on phylogenetic analysis of  $\beta$ -tubulin gene sequences**

**图 3 基于  $\beta$ -Tubulin 基因序列建立的系统进化树**

Note: The number at each branch points is percentage supported by bootstrap for 1 000 times. Date in parenthesis is GenBank accession number.

注：分支点上的数字表示 1 000 次 Bootstrap 重抽样分析的支持百分比；括号中的序号表示 GenBank 数据库中的登录号。

**Table 2 Frequency and colony forming unit per m<sup>3</sup> of *Penicillium bilaiae* in hospital air sample**  
表 2 医院样品中 *Penicillium bilaiae* 的分离频率和菌落形成单位/m<sup>3</sup>

SPECIES	Frequency (%)	CFU/m <sup>3</sup>
<i>Cladosporium cladosporioides</i>	26.5	9.0
<i>Penicillium polonicum</i>	23.5	8.0
<i>Penicillium bilaiae</i>	20.6	7.0
<i>Penicillium oxalicum</i>	8.8	3.0
<i>Penicillium sclertiorum</i>	5.9	2.0
<i>Penicillium purpurogenum</i>	2.9	1.0
<i>Pochonia chlamydosporia</i>	2.9	1.0
<i>Talaromyces flavus</i>	2.9	1.0
Total	100	34.0

studies on *Penicillium* in central air-conditioning systems. John C. S. Chang found out that dust accumulation and/or high humidity lead to *P. chrysogenum* growth in Heating Ventilation Air Conditioning (HVAC) duct materials<sup>[12]</sup>. This might partially explain why *P. bilaiae* was found in our samples. It should be paid enough attention about this habitat specificity of *Penicillium* which was first found in our research. And it was the dominant genus

in it.

Central air-conditioning system gives us convenience, while some healthy and safety concerns. By analyzing recent studies, Seppänen and Fisk found that there was an increase in prevalence of sick building syndrome (SBS) between 30% and 200% in the buildings with central air-conditioning system compared with natural ventilation system<sup>[13]</sup>. Many species of fungi may act as pathogens by means of invasive growth in human tissues, especially those of immunocompromised patients. They may cause inflammatory conditions through a variety of immunologic mechanisms<sup>[14]</sup>. If the central air-conditioning system is polluted by detrimental fungi in public places especially in hospitals, it might cause severe health issue. Previously, *Penicillium* as one of the most allergens has been reported. For example, a heat shock 70 protein isolated from *P. citrinum* has been identified as an allergen<sup>[15]</sup>. In this study, the pathogenicity of *P. bilaiae* was not assessed. Whether they will do harm to human health deserves more attention and research.

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