

Identification of *Penicillium* Species in Shiraz and vicinity

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Abstract

During 2006-2007, *Penicillium* species were collected from Shiraz vicinity, including Badjgah and Fars dairy factory (respectively 15 and 25km north of Shiraz). Fungi were isolated from contaminated culture media, rotted plant materials and pasturized cheese of Fars dairy factory. Hyphal tip isolates were grown on Czapeck yeast extract agar (CYA), malt extract agar (MEA) and 25% glycerol nitrate agar (G25N) and examined after seven days incubation at 25°C in the dark. Nitrogen and sugar consumption and indole production were also tested whenever necessary using creatine sucrose neutral agar (CSN) medium and Ehrlich reagent, respectively. Based on morphological and physiological criteria the following species were identified: *P. chrysogenum*, *P. citrinum*, *P. griseofulvum** and *P. waksmanii** (all from cheese), *P. aurantiogriseum**, *P. expansum* (from both cheese and contaminated media), *P. brevicompactum*, *P. citreonigrum**, *P. crustosum**, *P. roqueforti*, *P. solitum* and *P. viridicatum* (from contaminated media) , *P. italicum* (from citrus fruit) and *P. digitatum* (from kumquat fruit, in market, originated from north of Iran). The *Penicillium* species with asterisk are new to Iran flora.

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Introduction

Penicillium Includes some of the most economically important fungi. Biodeterioration and food spoilage, caused by some of the species of *Penicillium* are well known. Some species are toxigenic and can cause diseases in human and animals(12,13). On the other hand, some other species are useful in food and medical industry(18). Most *Penicillium* species are considered to be ubiquitous and opportunistic saprobes. A majority of taxons are soil fungi, accidentally occurring in food, but some are closely associated with food supplies.

Some species are specialized, such as fruit pathogens (e.g. *P. digitatum*, *P. expansum*, *P. italicum*, *P. oxalicum*, *P. solitum* and *P. ulaiense*) (5,14,16,20). A few grow at extreme environments such as low oxygen, low available water or low temperatures(18).

According to Pitt and Hocking(18), the genus *Penicillium* includes 150 species. In the past few years several techniques have been used together with traditional taxonomy based mainly on micro- and macromorphology, to establish natural groups in *Penicillium*. These techniques include the examination of secondary metabolites (3,4,10,11,23), volatile metabolites(6,7), cell wall polysaccharides (8), isozyme patterns (1), and DNA sequences (9,15,22).

A survey was conducted to identify *Penicillium* species in Shiraz area, in Fars province of Iran. The present work describes the species identified.

Material and Methods

During 2006-2007, *Penicillium* species were isolated from Shiraz vicinity, including Badjgah (15 km north of Shiraz) and Fars dairy factory (25 km north of Shiraz). Samples were isolated from

contaminated culture media, rotted plant materials and cheese. Hyphal tip isolates were grown on media suggested and identified by authentic keys (2,17,18,19) including Czapeck yeast extract agar (CYA), containing 0.1% K_2HPO_4 , 1% Czapeck concentrate, 0.0005% $CuSO_4 \cdot 5H_2O$, 0.001% $ZnSO_4 \cdot 7H_2O$, 0.5% yeast extract, 3% sucrose and 1.5% agar, pH 6.7; malt extract agar (MEA), containing 2% malt extract, 0.1% peptone, 2% glucose, 2% agar and 25% glycerol nitrate agar (G25N), containing 0.075% K_2HPO_4 , 0.75% Czapeck concentrate, 0.37% yeast extract, 25% glycerol and 1.2% agar. Czapek concentration contains 3% $NaNO_3$, 0.5% KCl, 0.5% $MgSO_4 \cdot 7H_2O$, 0.01% $FeSO_4 \cdot 7H_2O$.

(CSN) containing 1% CS concentrate, 1% sucrose, 0.5% creatine, 0.1% KH_2PO_4 , 0.005% bromocresol purple and 1.5% agar. CS (creatine sucrose) concentrate is composed of 5% KCl, 5% $MgSO_4 \cdot 7H_2O$, 0.1% $FeSO_4 \cdot 7H_2O$, 0.1% $ZnSO_4 \cdot 7H_2O$ and 0.05% $CuSO_4 \cdot 5H_2O$.

Ehrlich reagent is made by dissolving 4-dimethylaminobenzaldehyde (2g) in 96% ethanol (85 ml) and adding 10 N HCl (15 ml) (18).

Results and Discussion

Based on morphological and physiological criteria, 14 species of *penicillium* were identified as follows: *P. chrysogenum*, *P. citrinum*, *P. griseofulvum** and *P. waksmanii** (all from pasturized cheese), *P. aurantiogriseum**, *P. expansum* and (from both pasturized cheese and contaminated media), *P. brevicompactum*, *P. crustosum**, *P. citreonigrum** , *P. roqueforti*, *P. solitum* and *P. viridicatum* (from contaminated media), *P. italicum* (from citrus fruit) and *P. digitatum* (from kumquat fruit, in market, originated from northern Iran). Colonies on CYA and MEA after 7 days at 25°C, conidiophores (pencilli) and conidia of isolated species are shown in figures 1 to 14. A key is presented for identification of Shiraz *Penicillium* species (Table 1). With

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the exception of *P. digitatum*, *P. italicum*, *P. solitum* and *P. waksmanii*, all other isolated species are known to produce mycotoxins. The *Penicillium* species with asterisk are new to Iran flora.

Table 1. Key to *Penicillium* species isolated from shiraz vicinity, Iran.

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