First report of *Paecilomyces marquandii* from Iran

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Abstract

During 2008, the presence of *Paecilomyces* species was studied in fields in Fars province. Soil samples were collected from 0–20 cm depth. The isolates were recovered using a soil dilution plate method directly from soil. Isolation was performed from soil using malt extract agar, rice agar, oat-meal agar and potato dextrose agar. Anamorphic characteristics such as morphology of conidiophores, phialides, conidia and chlamydospores were investigated. Based on morphological and molecular characteristic the fungus was identified as *Paecilomyces marquandii*. The species is a new report for Iran. The isolates are kept in fungal collection of the Department of Plant Protection, Razi University, Kermanshah (Iran).

Keywords: Chlamydospore, conidia, Conidiophore, Fars province, morphology, phialide

نخستین گزارش از *Paecilomyces marquandii* برای ایران

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خلاصه

Paecilomyces یک قارچ هیفومیست با بیش از ۳۰ گونه شناخته شده می‌باشد. این جنس دارای زیستگاه‌های مختلفی از جمله خاک، مواد گیاهی پوسیده، مواد غذایی و حشرات می‌باشد. بعضی از گونه‌های این جنس باعث آلودگی انسان و حیوان می‌شوند. در سال ۱۳۸۷، حضور گونه‌های *Paecilomyces* در مزارع مختلف استان فارس بررسی شد. نمونه‌های خاک از عمق ۲۰-۰ سانتی‌متری جمع‌آوری، جداسازی از خاک با تهیه رقت و استفاده از محیط کشت‌های مالت آگار، برنج آگار، آرد یولاف آگار و سیب زمینی دکستروز آگار صورت گرفت. ریخت‌شناسی کنیدیوفور، فیالید، کنیدیوم و کلامیدوسپورها مورد بررسی قرار گرفت. همچنین نواحی ITS دی ان آ ریبوزومی هسته نیز در جدایه‌های خالص‌سازی شده توالی‌یابی شد. بر این اساس، جدایه‌های خالص‌سازی شده از جنس *Paecilomyces* تحت گونه *P. marquandii* تشخیص داده شدند که گزارش جدیدی برای فلور قارچی ایران می‌باشد.

واژه‌های کلیدی: استان فارس، ریخت‌شناسی، فیالید، کلامیدوسپور، کنیدیوفور، کنیدیوم، هیفومیست

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Introduction

Paecilomyces is a hyphomycetous fungus with more than 30 recognized species. *Paecilomyces* has different habitats comprised of soil, decaying plants residues, food products and insects. Some *Paecilomyces* species cause infections in humans and animals. Many environments including homes naturally have some *Paecilomyces* species, and this fungus does not generally cause deleterious health effects, except in people with compromised immune systems. Superficially, *Paecilomyces* fungi can resemble *Penicillium* molds, but these two genera are quite different. These fungi form filament-like structures, spreading to create a colony of powdery mold which can have a texture similar to that of suede or velvet in some species. Unlike the physically similar *Penicillium*, *Paecilomyces* does not develop greenish colonies. Some species can develop a sweetish smell, especially in the case of mature colonies. Several species are thermophilic, meaning that they thrive in high temperatures. These fungi are interesting to humans for a number of reasons. The first is that many prey on nematodes, which means that *Paecilomyces* can be used as a form of natural pest control. The fungi colonize the bodies of nematodes, eventually killing them with an overload of toxins (Khan *et al*. 2003). Some species also attack insects such as flies, gaining entrance to the body through one of the orifices such as the mouth. In humans, *Paecilomyces* can sometimes cause mycoses, fungal infections of the body, usually in the case of people with a weakened immune system. The fungus is cultivated as an alternative to wild *Cordyceps* fungi.

Materials and Methods

- Isolation

During 2008, the presence and frequency of ascomycetous fungi was studied in various fields in Fars province. In each location, samples were collected from 0–20 cm depth and passed through 2 mm, 40 and 60 mesh sieves. Using soil plating method, 10 g of soil samples were placed in 90 ml of 0.1% water-agar containing 100 ppm NPX (nonyl phenyl polyethylene glycol ether containing a concentration of 10.5 moles of ethylene oxide), mixed and serially diluted to 10^{-2} to 10^{-5} and 1 ml of each solution flooded on potato dextrose agar (PDA) and malt extract agar (MEA) by a L shape rod. These media were amended with Rose Bengal (45 µg/ml) and chloramphenicol (25 µg/ml). Plates were incubated at 25–27º C for 3–5 days for colony development. Individual strains were subcultured to new Petri dishes containing MEA and incubated at 25° C in the dark. The macroscopic features of the colonies and details of microscopic structures were observed using light microscopes. All isolates were identified at the species level.

- DNA extraction

For DNA extraction, isolates were grown on PDA for 10–15 days at 25° C in the dark. Fungal mycelium from pure cultures were scrapped and mechanically disrupted by grinding to a fine powder with liquid nitrogen using a mortar and pestle. Total genomic DNA was extracted using a genomic DNA purification Kit (Fermentas, UK) according to the manufacturer's instruction. The resulting DNA extracts were quantified by a NanoDrop spectrophotometer (NanoDrop Technologies, USA). DNA samples were kept at –20° C until they were used for PCR amplification.

- DNA amplification

The ITS regions of nuclear rDNA were amplified with the universal ITS1 (5´-TCC GTA GGT GAA CCT GCG G-3´) and ITS4 (5´-TCC TCC GCT TAT TGA TAT G-3´) primers (White *et al.* 1990) on a Corbett Research Model CG1-96 thermocycler. For ITS amplifications the samples were prepared as follows: a reaction tube contained, 12.5 µl diluted DNA sample (1:10 or 1:100 dilutions of the original extract), 2.5 µl of 10× PCR buffer, 20 pmol of each primer, 1.25 nmol of each deoxynucleotide, 1.5 mM of $MgCL_2$ and 0.5 U of *Taq* polymerase (CinnaGen, Iran) in a reaction volume of 25 µl. The amplification was carried out using the following program: an initial denaturation step at 94° C for 3 min; then 30 cycles, consisting of denaturation (30 s at 94° C), annealing (30 s at 50° C) and extension

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(2 min at 72° C), and a final extension step of 10 min was allowed at 72° C before cooling or removing the tubes. Amplified fragments were visualized under UV light after electrophoresis on 1% agarose gels stained with ethidium bromide and run in 1× TBE buffer. Controls with no DNA were included in every set of amplification to check the DNA contamination in reagents and reaction buffers.

- Sequencing of the amplified ITS regions

The amplification products of all specimens were purified with GeneJET PCR purification Kit (Fermentas, UK) to remove excess primers and nucleotides. Sequencing reaction was performed on purified PCR products in forward and reverse orientation using the primers used for amplification (ITS1 or ITS4). The sequence was determined with an ABI prism 377 DNA sequencer according to the manufacturer's instruction. All DNA sequences of the ITS regions deposited at the National Center for Biotechnology Information GenBank (NCBI, http://www.ncbi.nlm.nih.gov/Entrez) (Bethesda, MD, USA).

Results

Two hundred fungal isolates were recovered from soil samples. The most common fungi isolated from most soil samples were *Aspergillus* and *Penicillium* species. Other fungi were less frequent. On PDA and MEA medium, all species of *Aspergillus* and *Penicillium*, plus other soil fungi (*Cladosporium*, *Rhizopus*, *Fusarium*, *Rhizoctonia*) were easily isolated. *Paecilomyces marquandii* were identified on PDA by the production of pink colonies at three days after inoculation at 25° C.

- Morphological identification

Colonies on malt-extract agar growing moderately fast, attaining a diameter of 3–5 cm within one weeks at 25° C. Colonies consisting of a dense felt with a floccose overgrowth of aerial mycelium, in fresh isolates sometimes producing short erect loose synnemata; at first white, becoming pale vinaceous to purple (Fig. 1A). Exudate usually diffusing into the surrounding agar, with age becoming yellow-brown. Odour was absent. Vegetative hyphae were hyaline, smooth-walled, 2.5–3.2 µm wide. Conidiophores were hyaline, smooth-walled, 50–300 × 2.5–3 µm, and consisting of verticillate branches with whorls of 2–4 phialides (Fig. 1B). Phialides 8–15 × 1.5–2 µm, consisting of a short cylindrical to ellipsoidal basal portion, tapering into a distinct neck, about 1–2 µm wide (Fig. 1D). Conidia in dry divergent chains, ellipsoidal to fusiform, smooth-walled, hyaline, pale vinaceous in mass, 2–3 × 1.5–2 µm (Fig. 1G). Chlamydospore-like structures, thin-walled, globose to ellipsoidal and 3.5 µm in diameter (Fig. 1E–F).

- Molecular study

All *Paecilomyces* isolates previously identified based on morphological and culture characters, were amplified using the primers pair ITS1 and ITS4. An amplicon of about 600 bp was obtained for all of the *Paecilomyces* isolates. Through Blast search in GenBank all DNA sequences of *Paecilomyces* isolates (accession numbers: JQ013001 to JQ013003) showed 100% homology with sequences previously identified as and *P. marquandii* deposited in GenBank.

Discussion

In this study, 200 fungal isolates were recovered from soil. Based on morphological characteristics, fungi commonly isolated from soil were identified as *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Rhizopus* sp., *Paecilomyces lilacinus*, *P. variotii* and *Clonostachys rosea*. Results showed that *Aspergillus* spp. and *Penicillium* spp. as two major species obtained from most soil. Morphological characters of *P. marquandii* isolates showed major agreement with this species characterized in monographs (Samson 1974). *P. marquandii* produced chlamydospore-like cells on MEA and PDA as reported by Samson (1974). All isolates of *P. marquandii* were distinguished from *P. lilacinus* by the yellow reverse and smooth walled, hyaline conidiophores. Moreover, chlamydospore-like cells are usually present. Both fungi are rather common soil hyphomycetes (Domsch & Gams 1972). *Paecilomyces lilacinus* differs from *P. marquandii* by its pigmented conidiophores, which are usually rough-walled, and the uncoloured or purple reverse.

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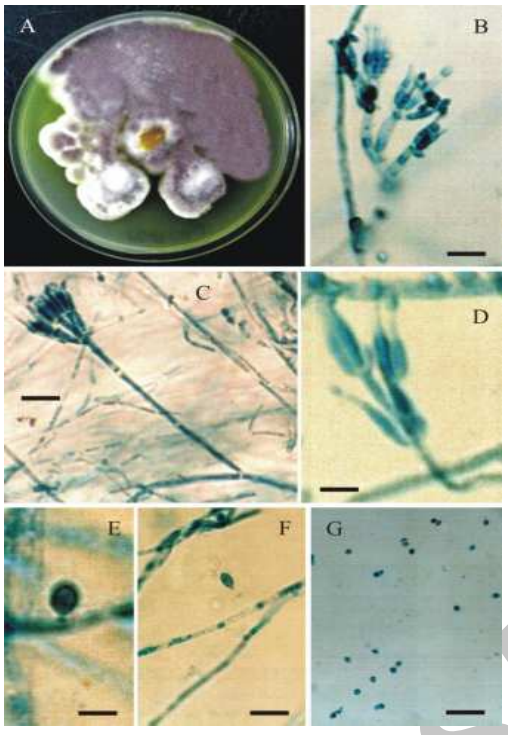

Fig. 1. *Paecilomyces marquandii*: A. Colony on MEA after 1 week at 25° C, B–D. Conodiophore and phialide, E–F. Chlamydospore, G. Spores (Bars: B–C & F–G = 16.7 µm, D–E = 10 µm).

Micro-morphological characters such as, size of spores, conidiophore and phialide morphology and cultural characters were found to be useful in distinguishing *Paecilomyces* species. But due to the overlapping in several characters among *Paecilomyces* species, some misidentification has been made when using these characteristics and seems that the use of molecular methods are needed for identification and separation of different species correctly. Using PCR with the primers ITS1 and ITS4, a fragment of about 600 bp was obtained for *P. marquandii* isolates. Based on ITS gene sequences, these isolates showed 100% homology with *P. marquandii* isolates deposited in GenBank. Subsequently, both phenotypical and molecular data confirmed the identification of the *Paecilomyces* isolates as *P. marquandii*. The type of conidia and chlamydospore-like cells and their dimensions agree with those described for *P. marquandii* isolates in other countries. This is the first report of *P. marquandii* with morphological and molecular details in Iran. Nevertheless, it seems that this species is less frequent compared with other *Paecilomyces* species such as *P. lilacinus* and *P. variotii*.

Previous studies showed that, *P. lilacinus*, *P. variotii*, *P. fumosoroseus* and *P. tenuipes* were present in Iran (Ershad 2009).

References

Domsch, K.H. & Gams, W., 1972. Fungi in agricultural soils. Longman, London.

Ershad, D. 2009. Fungi of Iran. Ministry of Jihad-e-Agriculture. 531 p.

Khan, A., Williams, K. & Nevalainen, H. 2003. Testing the nematophagous biological control strain *Paecilomyces lilacinus* 251 for paecilotoxin production. FEMS Microbiology Letters 227: 107–111.

Samson, R.A. 1974. *Paecilomyces* and some allied Hyphomycetes. Studies in Mycology 6: 1–119.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: PCR Protocols. A guide to methods and applications (M.A. Gelfand, D.H. Sninsky & T.J. White, eds) Academic Press, San Diego, CA: 315–322.

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