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Short communication

Rapid identification of *Ochroconis gallopava* by a loop-mediated isothermal amplification (LAMP) method

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Abstract

Ochroconis gallopava is a species of dematiaceous fungi recognized as a causative agent of zoonotic and emerging fungal infections. It affects the central nervous system and respiratory tracts of humans, birds and cats. We designed *O. gallopava* species-specific primer sets to aid in its identification by a loop-mediated isothermal amplification (LAMP) method based on the D1/D2 domain of the LSU rDNA sequence. The LAMP method successfully detected the gene from both fungal DNA and experimentally infected brains and spleens of mice and will be helpful in the diagnosis of *O. gallopava* infection.

Keywords: D1/D2 domain; Loop-mediated isothermal amplification (LAMP); *Ochroconis gallopava*; Phaeohyphomycosis; Zoonosis

1. Introduction

Ochroconis gallopava is a species of dematiaceous fungi recognized as a causative agent of zoonotic and emerging fungal infections (de Hoog et al., 2000). More than 30 human cases have been reported (Fukushima et al., 2005). The pathogen has caused outbreaks in poultry and wild birds, and a few cases in domestic cats (Kralovic and Rhodes, 1995). Environmental isolates of *O. gallopava* have also been found under low-pH and thermal conditions, such as in coal waste piles and hot springs, sewage from nuclear power plants, and broiler-house litter (Kralovic and Rhodes, 1995).

The disease in birds resembles the highly pathogenic H5N1 avian influenza (HPA) involving the central nervous system and respiratory tract. Therefore, a simple and rapid method of diagnosing *O. gallopava* infection is eagerly awaited because diagnosis by the isolation and identification of the fungus using mycological techniques is time-consuming and requires expertise.

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We recently developed the species-specific primer set of a highly pathogenic fungal species, *Paracoccidioides brasiliensis*, for use in a loop-mediated isothermal amplification (LAMP) method (Endo et al., 2004). The present study applied this method to the diagnosis of *O. gallopava* infection.

2. Materials and methods

2.1. Isolates

The species names *Diplorhinotrichum gallopavum*, *Dactylaria gallopava*, *O. gallopava*, *Dactylaria constricta* var. *galopava* and *Ochroconis gallopavum* are treated herein as *O. gallopava*, as proposed by de Hoog et al. (2000).

The *O. gallopava* isolates and related dematiaceous fungal species, and their genetic profiles of the divergent region of the D1/D2 domain of the large subunit of the rRNA gene (D1/D2 LSU rDNA) evaluated in the present study are shown in Table 1. Eight clinically important fungal species, *Aspergillus fumigatus* (IFM 40821), *Blastomyces dermatitidis* (IFM 41316), *Candida albicans* (IFM 5740), *Coccidioides immitis* sensu lato (IFM 50993), *Cryptococcus neoformans* (IFM 5830), *Histoplasma capsulatum* (IFM 41329), *Penicillium marneffei* (IFM 41708) and *Sporothrix schenckii* (IFM 47068), were also tested as negative controls.

2.2. Sequences for D1/D2 LSU rDNA

Fungal DNAs were extracted from cultures incubated on potato dextrose agar (PDA) slants (Difco, Franklin Lakes, NJ, USA) at 25 °C for 1–2 weeks. DNA was extracted with a DEXPAT® Kit (TaKaRa, Ohtsu, Japan) following the manufacturer's protocol with slight modification. Approximately 100 μl of fungal mass was transferred to a sterilized microtube (1.5 ml), and homogenized with 0.5 ml of DEXPAT® solution by a plastic pestle. The mixture was incubated at 100 °C for 10 min and centrifuged at 12,000 rpm (13,201 × *g*) for 10 min. The supernatant was used as the DNA sample (Sharmin et al., 2002). The sequence of D1/D2 LSU rDNA was processed by a standard method (Kurtzman and Robnett, 1997).

2.3. Species-specific PCR primer set for Ochroconis gallopava

A species-specific polymerase chain reaction (PCR) primer set for *O. gallopava* was designed based on the sequence of D1/D2 LSU rDNA of *O. gallopava* (accession number AB125281 in GenBank) with a comparison of 22 species of dematiaceous fungi obtained from the present study and from GenBank (*Cladophialophora bantiana*, AB100676; *Exophiala jeanselmei*, AB100664; *Exophiala spinifera*, AB100673; *Fonsecaea pedrosoi*, AB100632; *Phialophora verrucosa*, AB100610; *Rhinocladiella atrovirens*, AB091215). The primer sequences (Sigma-Genosys Japan, Ishikari, Hokkaido, Japan) were the following: OgF3: 5′-AGG GAG TCT CGG GTT AAG GG-3′ encoding from the 391st to the 410th, and OgB3: 5′-CAT TCC CTT CGT CTT TGT CC-3′ corresponding to the complementary sequence from the 718th to the 740th of AB125281.

PCR was carried out with the species-specific primer set under the following conditions. Approximately 20–40 ng of template DNA in 2.5 μl, one Ready-To-Go™ PCR bead (Amersham Pharmacia, Tokyo, Japan) and 10 pmol of primers OgF3 and OgB3 in 25 μl of total volume was subjected to an initial denaturing step of 4 min at 95 °C, 30 cycles of 1 min at 94 °C for DNA denaturation, 90 s at 58 °C for primer annealing, 2 min at 72 °C for primer extension, a final extension of 10 min at 72 °C, and was then maintained at 4 °C until electrophoresis. The PCR products from *O. gallopava* were sequenced by a method (Sharmin et al., 2002). The detection limits of the species-specific PCR for fungal DNA were evaluated in a serial dilution of DNA.

2.4. Loop-mediated isothermal amplification (LAMP) method

The specific primer sets for the LAMP method were as follows: OgF3, OgB3, FIP; 5′-ACT CGA CTC GTC GAA GGG GCA GAG GGT GAG AGT CCC GT-3′ designed from the forward sequence of 425th to 445th and the complementary sequence of 464th to 485th, and BIP; 5′-ACT GGC CAG AGA CCG ATA GCG TGA CTC TCT TTT CAA AGT GC-3′ designed from the forward sequence from 648th to 668th and the complementary sequence from 691st to 712nd of