NOTE Internal Medicine

Direct Detection of Dermatophytes in Skin Samples Based on Sequences of the Chitin Synthase 1 (CHS1) Gene

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ABSTRACT. For the direct detection of dermatophytes in skin scrapings and hairs from animals, a primer pair specific to the chitin synthase 1 (CHS1) gene of dermatophytes was constructed. By PCR analysis with the primer pair, dermatophyte DNA could be diagnosed directly and rapidly in clinical skin samples.

KEY WORDS: chitin synthase 1 gene, dermatophyte, PCR.


Dermatophytosis is common in animals and is sometimes transmitted to humans [4]. Diagnosis of this infection is usually performed by detecting fungal elements from the lesions and confirming the etiologic dermatophytes. To identify dermatophytes by these conventional methods, however, takes about one to two weeks to identify the dermatophyte. Therefore, a rapid detection method for dermatophytes in skin lesions is needed for diagnosing dermatophytosis.

In our previous study, in order to diagnose Microsporum canis infection rapidly, we amplified the chitin synthase 1 (CHS1) gene of M. canis in skin and hair samples from skin lesions on human and dogs with dermatophytosis by PCR analyses [1]. The sensitivity of this PCR system detection of one to two hundred fungal cells (10 pg of M. canis genomic DNA) [1]. However, the primer pair (primer 1 and primer 2) was also confirmed to amplify the CHS1 gene of other fungi such as Aspergillus species, Candida species and Malassezia species.

Therefore, we improved the PCR method and developed a more specific and more sensitive method for detecting dermatophyte DNA in skin scrapings from lesions.

The samples were taken from skin lesions of dogs, cats and a rabbit with dermatophytosis (Table 1). Microscopic examination of skin scrapings and hairs from the skin lesions revealed hyphae and arthroconidia. The samples were cultured on diluted Sabouraud’s dextrose agar [4] at 27°C for 7 to 10 days. The isolates were respectively identified as M. canis, M. gypseum and Trichophyton mentagrophytes by conventional analysis [4].

Table 1. Patients and microbiological tests

<table>
<thead>
<tr>
<th>Patients</th>
<th>Breed</th>
<th>Age</th>
<th>Sex</th>
<th>Direct exam.</th>
<th>Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dog</td>
<td>Pomeranian</td>
<td>10y*</td>
<td>Male</td>
<td>+*</td>
<td>M. canis</td>
</tr>
<tr>
<td>2. Dog</td>
<td>Beagle</td>
<td>10y*</td>
<td>Female</td>
<td>+</td>
<td>M. canis</td>
</tr>
<tr>
<td>3. Dog</td>
<td>Yorkshire Terrier</td>
<td>8y</td>
<td>Male</td>
<td>+</td>
<td>M. canis</td>
</tr>
<tr>
<td>4. Dog</td>
<td>Maltese</td>
<td>9y</td>
<td>Female</td>
<td>+</td>
<td>M. canis</td>
</tr>
<tr>
<td>5. Cat</td>
<td>Crossbreed</td>
<td>9y</td>
<td>Female</td>
<td>+</td>
<td>M. canis</td>
</tr>
<tr>
<td>6. Cat</td>
<td>Crossbreed</td>
<td>2m</td>
<td>Female</td>
<td>+</td>
<td>M. gypseum</td>
</tr>
<tr>
<td>7. Rabbit</td>
<td></td>
<td>3m</td>
<td>Female</td>
<td>+</td>
<td>T. mentagrophytes</td>
</tr>
</tbody>
</table>

a) y: Years. b): +: Microscopic examination of skin scraping and hairs from skin lesions revealed hyphae and arthroconidia. c) m: Months.
The PCR products were electrophoresed through 2% agarose gel and then stained with ethidium bromide. The about 450-bp PCR products were sequenced by the method of Kano et al. [1].

As a positive control for PCR analysis, we used the universal primers (primer1 and primer 2) for detecting CHS1 genes of many fungal species. The sequences of the degenerate primers for the CHS1 gene were based on the sequences reported in our previous study [2]. The PCR products were electrophoresed through 2% agarose gel and then stained with ethidium bromide.

The primer pair (CHS1 1S and CHS1 1R) amplified DNA fragments of about 450-bp from dermatophytes such as Epidermophyton floccosum, M. canis, M. gypseum, T. mentagrophytes and T. rubrum but not from the other pathogenic fungi (Table 2), bacteria, canine skin cells and feline skin cells (Fig. 2).

The primer pair (CHS1 1S and CHS1 1R) amplified the DNAs from skin scrapings and hairs from affected areas and produced about 450-bp fragments (Fig. 3). Also this primer pair did not amplify DNAs from the hairs from non-affected areas of patients and hairs from a healthy dog and a healthy cat (Fig. 3).

Amplification of DNA samples from the fungi including dermatophytes DNAs with universal CHS primers (primer 1 and primer 2) yielded fragments of about 620bp (Fig. 2).

The primer pair (CHS1 1S and CHS1 1R) amplified only dermatophyte CHS1 genes but not the other fungal CHS1 genes. Therefore, this molecular system should enable direct detection of dermatophyte DNAs in clinical samples.

More than 99% similarity was shown in the nucleotide sequences of the CHS1 gene between clinical samples.
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(patient no. 1 to 5) and a reference strain of M. canis (accession no. AB003564), clinical sample (patient no. 6) and a reference strain of M. gypseum (M. gypseum CHS1 gene accession no. AB003561), and a clinical sample (patient no. 7) and a reference strain of T. mentagrophytes (T. mentagrophytes CHS1 gene accession no. AB003558). Therefore, the CHS1 genes from the samples from the affected areas could be the CHS1 gene of M. canis, M. gypseum and T. mentagrophytes.

To examine the sensitivity of the PCR, the DNA from M. canis VUT-77054 was amplified with several concentration, 1 pg, 10 pg, 100 pg, 1 ng and 10 ng of template DNAs. PCR amplification with the primer pair could detect 1 pg of M. canis genomic DNA (Fig. 4).

This PCR system with the primer pair might be sensitive enough to detect ten to twenty fungus cells [5]. Therefore, the sensitivity of this system is 10 times higher with the dermatophyte specific primer pair than that used in the previous system [1]. These results suggest that the present method can rapidly and sensitively detect the specific DNA of M. canis, M. gypseum and T. mentagrophytes from clinical samples. Since infection due to these species is most common in dogs and cats, the method described here could be
useful for the clinical diagnosis of dermatophytosis in dogs and cats.

REFERENCES