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PCR Fingerprinting for Identification of Common Species of Dermatophytes

Classical identification of dermatophytes relies on morphological and physiological characteristics. The search for these characteristics often makes their identification long and laborious. In addition, phenotypic features can frequently vary from strain to strain or the organism can become devoid of distinguishing features.

In the last few years, genotypic approaches to identification have proven to be useful in solving taxonomic problems regarding dermatophytes. In fact, genotypic differences are considered more stable and more precise than phenotypic characteristics (2, 3).

In one of our preceding studies (1), we demonstrated the possibility of identifying various species of common dermatophytes and related fungi by PCR fingerprinting utilizing the simple repetitive oligonucleotide $(GACA)_4$ as a single primer (4). This primer appeared able to amplify all the strains that we tested and produced species-specific profiles for *Microsporum canis*, *Microsporum gypseum*, *Trichophyton rubrum*, *Trichophyton ajelloi*, and *Epidermophyton floccosum*, while intraspecific variability was not observed for these species. Three different profiles were observed in the *Trichophyton mentagrophytes* group (1).

We felt that the capacity of this primer to produce species-specific profiles together with the simplicity of the method and the reproducibility of the results (when one strictly maintains experimental conditions) could be exploited for identifying colonies that do not present species-specific morphological characteristics and are not identifiable with the classical methods (1). In particular, PCR fingerprinting could be useful for identifying the following: (i) very young colonies, (ii) colonies which have lost the morphological characteristics typical of the species, and (iii) dead strains.

To verify the first hypothesis, 12 strains from the Institut Pasteur collection (*M. canis* IP 2289-94 and IP 2145-93, *T. mentagrophytes* IP 1468-83 and IP 407-74, *Trichophyton interdigitale* IP 447-74 and IP 2190-93, *T. rubrum* IP 2360-96 and IP 2073-92, *M. gypseum* IP 2143-93 and IP 1463-83, and *E. floccosum* IP 1454-83 and IP 1559-84) were grown in Sabouraud's dextrose agar (SDA) (Difco) at 25°C; after 3 days, a colony (mean diameter, 5 mm) was taken and transferred to an Eppendorf tube containing 40 μl of sterile distilled water; the mycelium was homogenized with a manual homogenizer (Micro-Grinder; International PBI) for 1 min.

The Dynabeads DNA Direct System I (Dynal, Oslo, Norway) was used for the rapid DNA extraction. Briefly, 400 μl of Dynabeads was added to the homogenized mycelium, DNA was extracted as previously described (1), and the purity and quantity of the extract were determined spectrophotometrically (260 nm).

Template DNA (25 ng) was amplified using the $(GACA)_4$ primer, and PCR products were separated by electrophoresis and detected by ethidium bromide staining (1).

To verify the second hypothesis, we studied eight strains which had lost their typical morphological characteristics but which had originally been identified as *M. canis* (two strains), *T. rubrum* (four strains), or *T. interdigitale* (two strains) and came from both the collection of the Institut Pasteur (*M. canis* IP 2144-93 and *T. interdigitale* IP 406-72 and IP 2189-93) and our department's collection (five clinical isolates: one *M. canis* strain and four *T. rubrum* strains). When cultivated on SDA, these strains quickly developed white cottony-looking colonies that had none of the morphological characteristics (macroscopic and microscopic) of their species. Some aerial mycelium was taken from these colonies, DNA was extracted, and the PCR fingerprinting was done as described above.

We also studied 12 strains which upon subculture had not shown any growth. These old cultures had been grown 2 years previously and were preserved at room temperature in cork-closed SDA tubes. Except for two clinical isolates of *T. interdigitale*, which came from our department's collection, these nonviable strains came from the Institut Pasteur (*M. canis* IP 2145-93 and IP 1687-87, *T. mentagrophytes* IP 1468-83 and IP 401-69, *T. mentagrophytes* var. *granulosum* IP 1711-88 and IP 1182-79, *T. interdigitale* IP 102-77 and IP 447-74, and *E. floccosum* IP 1454-83 and IP 1559-84).

The DNA extracted from young colonies, the DNA extracted from strains that had lost their typical morphological characteristics, and the DNA extracted from nonviable strains were compared with the DNA extracted from cultures presenting typical morphological characteristics. This last DNA had been preserved at −20°C for 30 months after use in our previous study (1). The electrophoretic profiles of all the strains were perfectly superimposable on those of their own species which presented typical morphological characteristics.

In conclusion, the primer $(GACA)_4$ has produced species-specific profiles from fungi that are not identifiable with the classical techniques, and this further confirms that PCR fingerprinting could be of great help in the mycology laboratory in solving the many problems inherent in the identification of dermatophytes.

Although results with our technique proved to be extremely encouraging, we must caution that our results are restricted to a few species and strains of dermatophytes. Further investigation of a larger number of isolates could shed light upon possible interspecific relations.

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