Proposal of a new PCR-REA assay allowing for detection of pathogenic *Scopulariopsis* species

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Background

Fungi of the genus *Scopulariopsis* are environmental saprophytes being common in soil, air, and decaying plants but they have been also repeatedly isolated from household environments. Some species (at least nine: *S. acremonium*, *S. asperula*, *S. brevicaulis, S. brumptii*, *S. candida, S. carbonaria*, *S. flava*, *S. fusca* and *S. koningii*) are known to be opportunistic pathogens, being significant nondermatophytic causative agents of onychomycosis. Routine differentiation of pathogenic *Scopulariopsis* species according to phenotypic criteria is time-consuming and often inconclusive. Thus, there is a need for a rapid and a reliable molecular method allowing for inter- and intra-species differentiation of *Scopulariopsis* fungi.

The aim of this study was to develop a new PCR restriction-enzyme analysis (PCR-REA) assay for the identification of *Scopulariopsis* species based on the sequence analysis of partial *TUB* gene (coding for β-tubulin).

Materials and methods

Partial nucleotide sequences of the *TUB* gene (*ca*. 550 bp) were obtained for 76 strains, representatives of 30 different *Scopulariopsis* species (purchased from the Centraalbureau voor Schimmelcultures culture collection, Utrecht, the Netherlands) using degenerate primers, as described before [*Glass NL. and Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Appl Environ Microbiol. 6: 1323–30*]. Purified PCR amplicons were sequenced in both directions. Sequence data were assembled and analyzed with the EMBOSS package in terms of choosing restriction enzymes generating species-specific patterns. Designed method was then evaluated by performing digestion with selected enzymes on the amplicons representing partial *TUB* gene sequences obtained from all tested strains.

Results

Based on an *in silico* sequence analysis, two different PCR-REA assays with three different restriction enzymes (MfeI and SnaBI + TfiI) were designed, specific for the identification of four *Scopulariopsis* species including three clinically important (i.e. *S. brevicaulis, S. brumptii, S. asperula*, and *S. halophilica*) (**Table 1**). The results obtained with computer-aided analysis were identical to those obtained with enzymatic digestion and agarose gel electrophoresis (with an exception for one *S. acremonium* strain) (**Figure 1**).

Table 1. Differentiation of *Scopulariopsis* species with PCR-REA of the *TUB* gene using MfeI and SnaBI + TfiI digestion enzymes.

Species	Restriction enzyme	Fragments size [bp]
S. brevicaulis	MfeI	321, 223
S. halophilica	MfeI	418, 85
S. brumptii	SnaBI + TfiI	378, 106, 64
S. asperula	SnaBI + TfiI	209, 134, 134, 65

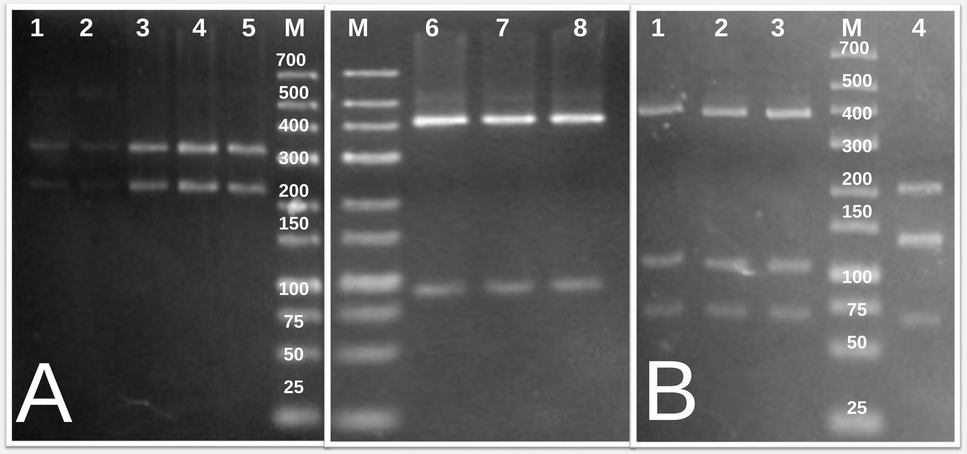

Figure 1. Results of *TUB* PCR-REA profiling for the identification of four *Scopulariopsis* species including three clinically important. **A:** Using MfeI enzyme, Lanes 1-5 - *S. brevicaulis*, 6-8 - *S. halophilica*. **B:** Using SnaBI + TfiI enzymes, Lanes 1-3 - *S. brumptii*, 4 - *S. asperula*. M - Molecular weight marker (GeneRuler LowRange DNA Ladder).

Conclusion

The present study offers a new molecular tool for the identification of *S. brevicaulis, S. brumptii, S. asperula,* and *S. halophilica.* Developed method involves PCR amplification of *ca*. 550-bp *TUB* gene fragment, followed by digestion with the MfeI and SnaBI+TfiI restriction endonucleases, and agarose gel electrophoresis. The proposed PCE-REA assay should provide a clinically useful method of identifying of the three pathogenic *Scopulariopsis* species.

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