

Title: 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 complicated. Thus, there is a need for a rapid and a reliable molecular method which allows 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).

Material/methods: Partial nucleotide sequence of the TUB gene (~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 was assembled and analyzed with the EMBOSS package in terms of choosing restriction enzymes generating species-specific patterns. Designed method was then evaluated with subsection to digestion with selected enzymes the amplicons representing partial TUB gene sequence obtained from all tested strains.

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

Conclusions: 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 electrophoresis on agarose gel. The proposed PCR-REA assay should provide a clinically useful method of identifying three pathogenic *Scopulariopsis* species.

Keywords: *Scopulariopsis* sp., onychomycosis, molecular identification, TUB