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 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).

Based on an *in silico* sequence analysis, two different PCR-REA assays with three different restriction enzymes (MfeI and SnaBI + Tfil) 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).

### 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

![Figure 1](image1.png)

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 + Tfil enzymes, Lanes 1-3 - *S. brumptii*, 4 - *S. asperula*. M - Molecular weight marker (GeneRuler LowRange DNA Ladder).

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Restriction enzyme</th>
<th>Fragments size [bp]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. brevicaulis</em></td>
<td>MfeI</td>
<td>321, 223</td>
</tr>
<tr>
<td><em>S. halophilica</em></td>
<td>MfeI</td>
<td>418, 85</td>
</tr>
<tr>
<td><em>S. brumptii</em></td>
<td>SnaBI + Tfil</td>
<td>378, 106, 64</td>
</tr>
<tr>
<td><em>S. asperula</em></td>
<td>SnaBI + Tfil</td>
<td>209, 134, 134, 65</td>
</tr>
</tbody>
</table>

### 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+Tfil 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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