Proposal of a set of PCR-RFLP assays for the identification of *Scopulariopsis* fungi of clinical importance

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Introduction and objectives

The genus *Scopulariopsis* accommodates more than 30 species of ascomycete mitosporic moulds, whose natural habitat is the soil, where they live as saprotrophs and assist in the decay of organic matter. However, some members of the *Scopulariopsis* genus may, under certain conditions, usually linked with host immunodeficiency, cause opportunistic infections in humans. At least 9 species (*S. acremonium*, *S. asperula*, *S. flava*, *S. fusca*, *S. carbonaria*, *S. koningii*, *S. brevicaulis*, *S. brumptii*, and *S. candida*) have been documented in the literature as the aetiological agents of human mycoses. Identification of pathogenic *Scopulariopsis* spp. still largely relies on the phenotype-based methods, which are laborious, time-consuming, and often result in misidentification. Therefore, molecular diagnostic methods are increasingly being developed to assist the identification of *Scopulariopsis* spp. However, none of the methods currently available permits inter- and intra-species differentiation of *Scopulariopsis* fungi.

The purpose of this study was to describe the genetic diversity of *Scopulariopsis* spp., based on the sequence analysis of partial *COX1* and *TUB* genes, coding for cytochrome c oxidase subunit 1 and β-tubulin, respectively, and to use the obtained data to design PCR-restriction fragment length polymorphism (RFLP) assays allowing the identification of *Scopulariopsis* spp.

Materials and methods

The study included 78 strains, being representatives of 30 different *Scopulariopsis* species, and purchased from the Centraalbureau voor Schimmelcultures (CBS) culture collection, Utrecht, the Netherlands. Partial *COX1* and *TUB* genes were PCR-amplified, using specially designed degenerate primers, and sequenced in both directions. The sequence data were analysed using ClustalX, and the phylogenetic trees were constructed with MEGA6 by using the Maximum Likelihood method. The choice of restriction enzymes generating species-specific patterns, and the RFLP assays were performed *in silico* using the programs of the EMBOSS package.

Results

The sequence analysis of both *COX1* and *TUB* partial genes demonstrated a high degree of intra-species diversity. Within each species, the level of *COX1* and *TUB* gene sequence similarity ranged from 79.5-99.3% and from 76.3-100%, respectively. Based on a comprehensive bioinformatic sequence analysis, five different PCR-RFLP assays were developed (two for the *COX1* gene and 3 for the *TUB* gene), specific for 5 clinically important *Scopulariopsis* species (*S. brevicaulis, S. brumptii, S. koningii S. acremonium*, and *S. carbonaria*).

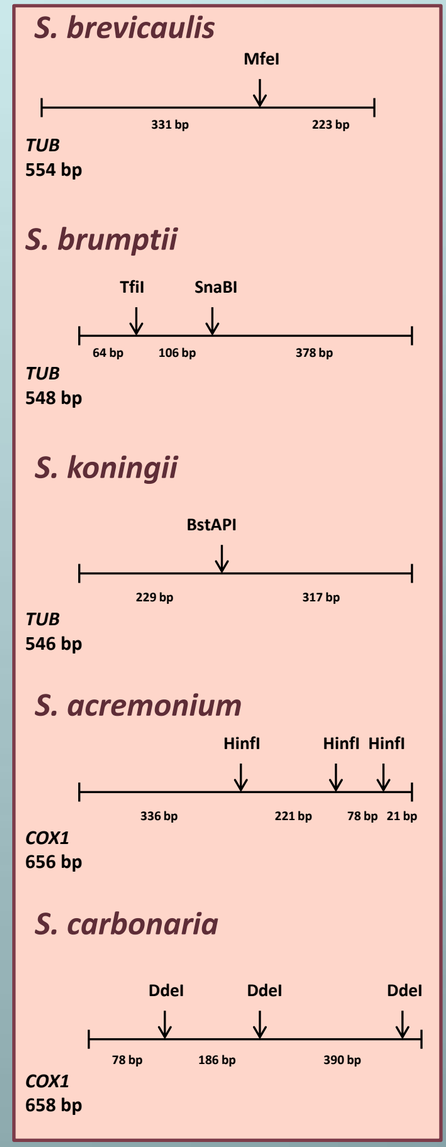

Fig.1 RFLP assays generating specific profiles for five species of clinical significance. The scale was not maintained.

Conclusions

This study revealed a high genetic diversity of *Scopulariopsis* spp. Five species of clinical importance could be separated using 5 different PCR-RFLP assays of the *COX1* and *TUB* partial genes. The results of the sequence analysis of these genes indicated certain inaccuracies in species identification of some of the *Scopulariopsis* strains deposited in the CBS database and used in this study.

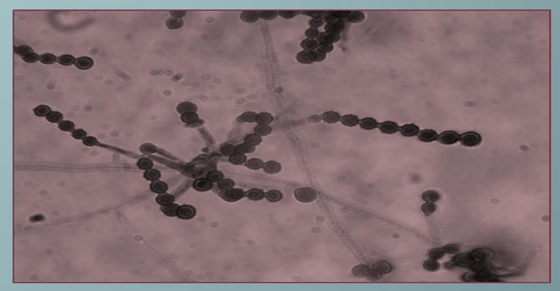

Fig. 2 Conidiophores of *Scopulariopsis brevicaulis*. (Photo by M. Skóra)

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