Evaluation of species diversity among Indian clinical *Schizophyllum commune* using matrix-assisted laser desorption ionization–time of flight mass spectrometry and MLST

Pradeep Kumar Singh¹, Jacques F. Meis², Anuradha Chowdhary^{1*}

¹Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India; ²Radboudumc/CWZ, Nijmegen, Netherlands

Objectives

- *Schizophyllum* is an important plant and animal pathogen in the genus basidiomycetes.
- *Schizophyllum commune* is the best-known species causing allergic and invasive mycoses since 1950.
- Key morphological features necessary for the recognition of basidiomycetes fungi are the presence of clamp connections on hyphae and the development of fruiting bodies.
- However, the final species identification needs confirmation by molecular methods.
- The aim of this study was to determine the species diversity among Indian clinical isolates of *Schizophyllum* using multilocus phylogenetic analysis and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS, Bruker Biotyper).

Methods

- **Isolates:** Thirty-seven molecularly identified clinical isolates of *S. commune* were selected from the culture collection of Medical Mycology, V. P. Chest Institute, Delhi, India, to determine the species diversity along with the reference isolates of *S. commune* (CBS 333.85, CBS 579.83, CBS109426 and CBS10320) and *S. radiatum* (CBS 301.32).
- **MLST:** The markers used for sequencing were the internal transcribed spacer (ITS), a portion of the nuclear large subunit (LSU) of ribosomal DNA, the RNA polymerase II second-largest subunit (*RPB2*), and the translation elongation factor 1 α (*EF-1 α*) gene. The neighbor joining (NJ) phylogenetic tree was constructed using MEGA (v.6) software.
- **MALDI-TOF MS:** The isolates were also processed for identification and to upgrade the in-house database by creating the main spectrum (MSP) using MALDI-TOF MS.

Results

- The LSU, *EF-1 α* , and *RPB2* markers showed consistency and concatenated sequences were analyzed. The analyses revealed two main clades in the phylogenetic tree.
- One clade included all the Indian and CBS reference strains of *S. commune* (n=38), and the other clade included only reference strain of *S. radiatum* (CBS 301.32) used in the study.
- The reference strains of *S. umbrinum* and *S. fasciatum* acted as outgroups since the genetic distance to the clades of *S. radiatum* and *S. commune* were elevated.
- MALDI-TOF MS showed all the isolates as *S. commune* including *S. radiatum* reference strain as the current Bruker database does not contain MSPs for *S. radiatum*.

Results

- MALDI-TOF MS correctly identified all the isolates after the upgradation of database.
- MSP dendrogram also showed two different clusters for *S. commune* and *S. radiatum*, which were in concordance with the NJ phylogenetic tree based on concatenated sequences.

Figure: (A) Score-oriented dendrogram of the main spectra of *Schizophyllum commune* and *S. radiatum*; (B) Neighbour joining tree obtained from the combined LSU, *EF-1 α* , and *RPB2* sequences of the isolates. Bootstrap support values over 70 are indicated on the nodes.

Conclusion

- The importance of *Schizophyllum* in the clinical setting has been demonstrated by the number of reported cases and strains isolated.
- In Indian scenario, *S. commune* is the prevalent species in allergic and invasive pulmonary infections instead of *S. radiatum* which is most frequently recovered species from USA.
- *EF1* and *RPB2* alone can differentiate between these two species of *Schizophyllum* and thus the two markers can be used for the identification of *Schizophyllum* species.
- MALDI-TOF MS plays an important role in rapid identification of *Schizophyllum* species as it takes only half an hour for the identification but it has limited database. Creation of in-house database can improve the identification rate drastically.