Molecular Identification of Non-Sporulating Moulds Isolated from Patients with Fungal Sinusitis

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OBJECTIVE

Morphological identification of moulds using traditional culture methods depends largely on the development of characteristic fruiting structures. However, a proportion of moulds do not display such features despite much efforts in attempting to encourage sporulation using various laboratory methods. In addition, clinical significance of these non-sporulating moulds is difficult to determine, especially when they were recovered from non-sterile sites and the identity is unknown. The objective of this study is to examine the utility of using molecular sequencing in identification of non-sporulating moulds and their significance when isolated from patients with chronic rhinosinusitis.

METHODS

Five moulds were isolated in 2017 from tissue and pus obtained from the nasal cavity or maxillary sinus in patients with chronic rhinosinusitis. They were isolated and subcultured onto Potato Dextrose Agar at 30°C and observed for sporulation for 3 weeks. They could not be identified due to the lack of distinctive features at the end of incubation period. These moulds were sent for further workup using molecular sequencing. Briefly, DNA were extracted and PCR was performed using primers targeting ITS, D1/D1 region of the 28s ribosomal subunit and, where necessary, beta-tubulin gene. Amplified products were purified, sequenced and compared with available sequences in NCBI.

RESULTS

The results of 3 non-sporulating moulds showed *Aspergillus fumigatus* complex :

- Identified using sequences from ITS, D1/D2 regions and beta-tubulin gene.
- None of the three displayed morphological features that were typical of *Aspergillus* species; white woolly growth with septated hyaline hyphae were observed.
- Failed to grow at 42°C.
- In two of the cases where tissue samples were sent for histological examination, presence of mycetoma was reported, supporting the significance of *A. fumigatus* complex isolated.

The other two moulds showed *Schizophyllum commune* :

- Sequencing was performed from ITS and D1/D2 regions.
- One case presented clamp connections, spicules and basidiospores in Lactophenol Cotton blue (LPCB) wet mount. Basidiocarps were only observed from the culture plates after 4 weeks of exposure to light and dark conditions. (See images)
- Histology showed fungal elements with eosinophilic collections.
- Only hyaline and septated thin hyphae were seen from white woolly moulds for both cases.
- No pronounced odour.

Schizophyllum commune on Potato Dextrose Agar

Microscopic image of *Schizophyllum commune*

CONCLUSION

Identification of non-sporulating moulds using phenotypic characteristics has always been challenging for most mycology laboratory, and failure to fully identify the moulds could potentially affect management in certain clinical scenario. Molecular sequencing has enabled the laboratory to successfully identify all non-sporulating moulds isolated from patients with chronic sinusitis. Coupled with histological findings, molecular sequencing has assisted the laboratory in determining the significance of such moulds and differentiating them from other fungal colonisers.

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