Species identification of Aspergillus isolates by DNA sequencing.

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Introduction

Aspergillus species are associated with a variety of invasive and cutaneous infectious diseases, from which infections of the bronchopulmonary system, ear canals and sinuses are the most common forms. Phenotype-based identification of Aspergillus allows recognizing isolates to species level. DNA sequence-based identification can demarcate species within the complexes and reveal cryptic species, that are not possible to identify using classical methods and frequency of which is not known at present time.

Objectives

The objective of this study was to characterize spectrum of Aspergillus species in clinical isolates collected in Saint Petersburg area (Russia) using morphological criteria and sequence analysis of rDNA and β-tubulin gene fragments.

Materials and Methods

167 Aspergillus isolates from the Russian Collection of Pathogenic Fungi (Kashkin Research Institute of Medical Mycology)

| 137 isolates from clinical specimens |
| 30 isolates from environmental sources |

1. Morphological identification
2. Sequencing
3. BLAST
4. Phylogenetic analysis by MEGA 5.03 software

BLAST: The identification to species level was concluded at 99-100% sequence identity.

Results

Sequence-based identification to the species level

β-tubulin - 127 (76%) isolates were unambiguously identified
ITS1-5.8S-ITS2 - 83 (50%) isolates were unambiguously identified

For precise species identification of 19 (11%) ambiguously identified isolates phylogenetic analysis of their β-tubulin sequences and β-tubulin sequences of the type strains available in GenBank [1,2] was performed.

A. awamori
Isolates with intermediate genotype
A. niger

Fig. 4 Neighbor Joining phylogenetic tree of tubulin fragment of A. niger species complex isolates. Sequences of type strains [1] are marked by green circles.

Phylogenetic analysis of the "niger" group (fig. 4) revealed isolates with an intermediate genotype between A. awamori and A. niger types.

Conclusions

1. In most cases, β-tubulin locus sequencing can adequately discriminate Aspergillus species, including cryptic species. We have revealed 7 isolates that genetically correspond to an intermediate variant between A. awamori and A. niger.
2. The most common species associated with invasive aspergillosis of lung was A. fumigatus, with otomycoisis - A. niger and A. awamori, with nasal aspergillosis – A. flavus.

References: