

ORT_17 - Molecular identification of filamentous fungi in a pharmaceutical industry by sequencing the D2 domain from ribosomal DNA

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Introduction: Filamentous fungi are among the main groups of microorganisms contaminating products in the pharmaceutical industries. The D2 domain of ribosomal deoxyribonucleic acid (rDNA) allows the analysis of polymorphism in isolates and it is applied to taxonomic classification of fungal species.

Objective: This study aimed to evaluate the use of the D2 domain of rDNA in the molecular identification of fungal isolates in a pharmaceutical industry.

Methodology: Twenty-eight filamentous fungi strains isolated between 2019 and 2021 from: environmental monitoring samples (n = 18), bioburden analysis of solutions (n = 4), water samples (n = 2), culture medium and cell line used in production (n = 2), sterility test of intermediate product (n = 1) and cell line used in quality control testing (n = 1) were analyzed. The 28 strains were cultivated on Potato Dextrose Agar and identified by sequencing using the MicroSEQ® D2 rDNA kit, according to the manufacturer's instructions, which provides sequences with 300 to 500 pairs of bases (bp). The sequences obtained were assembled with BioNumerics software v. 8.1 and the contigs analyzed in database using the Basic Local Alignment Search Tool (BLAST). A similarity value >97% was considered for species identification. The Simpson's index (SI) was applied to calculate the resolution power of D2 domain sequencing for genus identification.

Results: The 28 strains presented an average of 314 bp and more than 97% similarity in the analysis. The SI calculated was 0.83. The strains were identified: *Penicillium* spp. (n = 6), *Cladosporium* spp. (n = 6), *Aspergillus* spp. (n = 3), *Cladosporium endophyticum* (n = 3), *Chaetomium* spp. (n = 2), *Fusarium incarnatum* (n = 1), *Cladosporium cycadicola* (n = 1), *Coniochaeta mutabilis* (n = 1), *Diaporthe* spp. (n = 1), *Scopulariopsis alboflavescens* (n = 1), *Microdochium seminicola* (n = 1), *Neurospora* spp. or *Sordaria* spp. (n = 1), *Subramaniula* spp. or *Allobostryotrichum* spp. or *Chaetomium* spp. (n = 1).

Conclusion: The D2 sequencing analysis was sufficient to identify most of the strains at least at the genus level (n = 26, 92.9%), and eight (28.6%) strains were identified at species level. Two (7.1%) strains presented more than one possible genus, so new studies are necessary in order to identify these strains at species level, as ITS sequencing.

Keywords: Filamentous fungi; Molecular identification; rDNA