

6-1 MOLECULAR IDENTIFICATION OF FILAMENTOUS FUNGI IN A PHARMACEUTICAL FACILITY BY SEQUENCING OF THE INTERNAL TRANSCRIBED SPACERS (ITS) OF RIBOSOMAL DNA

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Resumo:

Filamentous fungi are widely present in nature, being considered one of the main sources of contamination in pharmaceutical production areas. The molecular identification of filamentous fungi is an important tool in the development of strategies aiming at the reduction of productivity loss, batch failure and quality deviations. The internal transcribed spacers (ITS) regions have been frequently used in the identification of fungi because they have accumulated more mutations over time than the coding regions, which allows differentiating species and estimating the divergence time between taxa. This study aims to evaluate the ITS region in the molecular identification of fungi isolated in a pharmaceutical industry. Fifty filamentous fungal strains isolated between 2019 and 2021 were selected from samples of: environmental monitoring (n=34), sterility test of intermediate products (n=4), bioburden analysis (n=4), water analysis (n=3), aseptic simulation (n=3), culture medium (n=1), and cell lineage used in production (n=1). The strains were grown on Potato Dextrose Agar and the extracted DNA was amplified and sequenced using the universal primers ITS1 and ITS4, providing sequences ≥ 500 base pairs (bp). The sequences obtained were assembled and the contigs analyzed in the Genbank using the Basic Local Alignment Search Tool. The thresholds considered for species, genus and family identification were $\geq 99.6\%$, $\geq 94.3\%$ and $\geq 88.5\%$, respectively. The Simpson's index (SI) was applied to calculate the resolution power of ITS sequencing for genus identification. The average size of the 50 sequences was 565 bp. The calculated SI was 0.764. The strains were identified as: *Penicillium spp.* (n=13), *P. meleagrinum var viridiflavum* (n=2), *P. citrinum* (n=2), *P. camerunense* (n=1), *P. coeruleoviride* (n=1), *Aspergillus spp.* (n=6), *A. sydowii* (n=7), *Chaetomium spp.* (n=2), *Cladosporium spp.* (n=4), *C. halotolerans* (n=2), *C. endophyticum* (n=1), *C. sphaerospermum* (n=1), *Coniochaeta fasciculata* (n=1), *Curvularia spp.* (n=1), *Diaporthe spp.* (n=1), *Fusarium spp.* (n=1), *Chaetomiaceae* (n=1), *Microdochium spp.* (n=1), *Rhizopus spp.* (n=1), *Trichoderma longibrachiatum* (n=1). Sequencing analysis of the ITS region was sufficient to identify 30 (60%) strains at the genus level, 19 (38%) at the species level and one (2%) at the family level. The ITS region was able to identify most filamentous fungal strains at least at the genus level and seems to be an alternative method for fungi identification in the Pharmaceutical Industry.

Palavras-chave:

filamentous fungi, internal transcribed spacer, pharmaceutical facility, genetic sequencing, microbial identification

Agência de fomento:

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