Identification and Molecular Typing of Clinical Fusarium Isolates by Two DNA Sequencing Methods and Automated rep-PCR

ABSTRACT

Fusarium species are increasingly recognized as human pathogens. We recently described the use of automated rep-PCR for the identification of Fusarium species. In order to evaluate the utility of automated rep-PCR for the identification of Fusarium species, we performed an additional analysis of 21 clinical isolates to the species level using automated rep-PCR. For each of the 21 isolates, we sequenced the ITS1-5.8S-ITS2 regions of the 28S rDNA gene and the EF1-α gene region. The results were analyzed using the Diversilab software v2.1. We obtained rep-PCR fingerprints for each isolate and compared the fingerprints with the rep-PCR fingerprints of known Fusarium species. The results indicated that the Diversilab System can be a useful tool to differentiate Fusarium species and the approach shows strong promise for studying the epidemiology of fusariosis.

BACKGROUND

Fusarium species are emerging and severe opportunistic mold pathogens, in particular, Fusarium oxysporum and Fusarium solani. These species are the third most common fungal genus (after Candida and Aspergillus) isolated from systemic infections in bone marrow transplant patients. Most of the infections are caused by species. 7, F. verticillioides (F. nivale), and F. solani, each responsible for about 35% of clinical cases. The diagnostic and treatment strategies are based on morphology and epidemiology. These traditional methods of identification for Fusarium species are time-consuming and only well-trained mycologists are able to diagnose the species at the genus level.

METHOD

We tested 21 clinical Fusarium species isolates recovered from cancer patients as determined by morphological analysis along with 5 ATCC clinical isolates. Species identification of each sample was performed using the NovaTool Identification Kit (Spectral Genomics, Inc) for DNA fingerprinting. The amplicons of 15 samples were sequenced using molecular technology on the ABI 3730 (Applied Biosystems, Inc) and the results were analyzed by the Diversilab software v2.1 (Fig 2). Each species was additionally sequenced by both the 28S DNA gene region and the EF1-α gene region. Two separate amplicons were generated using ITS1 and ITS2 primers and EF1-α primers. The amplicons were purified using High Pure PCR Product Purification Kit (Roche Diagnostics Co, IN) and the subsequent sequencing was performed with the SeqSight Terminator.1 curve Sequencing Kit (Applied Biosystems, CA) along with ITS1 and EF1-α PCR primers. The products were purified using PERKEM ET Gel Filtration Kit (Pharmacia Biotech, CA) and sequenced using BigDye Terminator (Applied Biosystems, CA). The results were analyzed using Sequencing Analysis 3.1. The BLAST results were obtained using the NCBI BLAST ( press the BLAST button) and identified using BLAST on the NCBI website (www.ncbi.nlm.nih.gov/BLAST/). The EF1-α gene region was amplified on the Fusarium Diversilab (Spectral Genomics, Inc). The rep-PCR analysis was performed on the Fusarium Diversilab (Spectral Genomics, Inc). The results of the rep-PCR analysis were compared with the rep-PCR fingerprints of known Fusarium species. The results indicated that the Diversilab System can be a useful tool to differentiate Fusarium species and the approach shows strong promise for studying the epidemiology of fusariosis.

RESULTS

The Diversilab System was used to generate rep-PCR DNA fingerprints for each isolate and the similarity compared to known species was shown as a dendrogram for each clinical isolate. The rep-PCR fingerprints were generated using the Diversilab System and the similarity compared to known species was shown as a dendrogram for each clinical isolate. The results indicated that the Diversilab System can be a useful tool to differentiate Fusarium species and the approach shows strong promise for studying the epidemiology of fusariosis.

REFERENCES


Fig 1. Characteristics of Fusarium species and the species discrimination observed in the rep-PCR patterns was expected due to the presence of virulence factors. These results support rep-PCR as a possible method for identification of Fusarium species. We present the use of a commercial automated rep-PCR assay system for the rapid and accurate identification of Fusarium species and the potential to obtain strain-level differentiation.