Molecular fingerprinting of clinical Candida isolates using automated rep-PCR


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Candidiasis has become a more common, life-threatening infection. In the past, these infections were due primarily to C. albicans. However, recent reports have indicated non-albicans infections are on the rise, often accounting for more than 50 percent of candidiasis found in the infected population. Additional drug resistance may vary by species. Thus, factors increase the need for a rapid and accurate method of identification for Candida species. Traditional automated and biochemical systems for Candida species provide identification only at the genus level and may take several days to perform. Therefore, these biochemical tests have been identified as insufficient for identifying clinical isolates. Current molecular methods, generally for strain typing, may be less automated (PFGE), tedious (ribotyping), or require specific instrumentation (RAPD). Previous reports describe the application of a repetitive sequence-based PCR (rep-PCR) method for bacterial strain typing and for characterizing the genotypic relatedness among Candida species. These findings support the potential of a commercially automated rep-PCR assay system, the DiversiLab System, for use in rapid fungal identification and strain-level differentiation. This study explored the performance of the DiversiLab System as a genotyping tool for identifying clinical Candida isolates.

**BACKGROUND**

Sixty-three characterized Candida isolates (10 ATCC and 53 clinical) including 26 C. albicans, 10 C. parapsilosis, 9 C. glabrata, 4 C. tropicalis, 3 each of C. krusei, C. dublinensis, and C. dublinensis, 1 each of C. lusitaniae, C. gilvogrisea, C. haemulonii, C. famata, and C. nonafinis were grown on TSA II enriched with 10% sheep’s blood. Genomic DNA was extracted using the DiversiLab system's V1 kit for DNA fingerprinting (Bacterial Barcodes, Inc.). The amplified product (1 µl) was separated and detected by agarose gel electrophoresis (Agilent Technologies, Inc). The results were analyzed by comparing the samples to the DiversiLab DNA fingerprinting library available with the DiversiLab software. The analysis utilized the DiversiLab software (v2.16), which uses Pearson’s correlation coefficient for similarity calculation and unsupervised pairwise grouping with mathematical averaging (UPGMA) for creating reports. Discordance sample identification was resolved by ITS1 -5.8S-ITS2 region sequencing. A previously published method that utilizes unweighted pairwise grouping with mathematical averaging (UPGMA) for creating reports. Discordant samples were grown on TSA II enriched with 10% sheep’s blood. Genomic DNA was extracted using the DiversiLab system’s V1 kit for DNA fingerprinting (Bacterial Barcodes, Inc.). The amplified product (1 µl) was separated and detected by agarose gel electrophoresis (Agilent Technologies, Inc). The results were analyzed by comparing the samples to the DiversiLab DNA fingerprinting library available with the DiversiLab software. The analysis utilized the DiversiLab software (v2.16), which uses Pearson’s correlation coefficient for similarity calculation and unsupervised pairwise grouping with mathematical averaging (UPGMA) for creating reports. Discordance sample identification was resolved by ITS1 -5.8S-ITS2 region sequencing. A previously published method that utilizes unweighted pairwise grouping with mathematical averaging (UPGMA) for creating reports. Discordant samples, as well as those in the clusters in their libraries, were sequenced utilizing the ITS1 -5.8S-ITS2 region. The results confirmed rep-PCR clustering by identifying sample 47C. tropicalis and sample 58 and 59C. dublinensis.

**METHOD**

The sixty-three isolates (red circles) were compared against the V1 rep-PCR fingerprint (Figure 1). Using the DiversiLab System, identification was based on clusters with average similarities greater than 95%. When compared to biochemical testing for C. dublinensis identification, the DiversiLab system was 95% concordant. Samples 47 and 48 were discordantly identified as C. dublinensis; however, a different sample (rep-PCR clustered sample 47C. tropicalis and sample 48 and 49 and samples 58 and 59C. dublinensis (with sample 60). The discordant samples, as well as the samples in their clusters, were sequenced utilizing the ITS1 -5.8S-ITS2 region. The results confirmed rep-PCR clustering by identifying sample 47C. tropicalis and sample 58 and 59C. dublinensis.

**RESULTS**

The sixty-three isolates (red circles) were compared against the V1 rep-PCR fingerprint library (Figure 2). Using the DiversiLab System, identification was based on clusters with average similarities greater than 95%. When compared to biochemical testing for C. dublinensis identification, the DiversiLab system was 95% concordant. Samples 47, 58, and 59 discordantly identified as C. dublinensis; however, rep-PCR clustered sample 47C. tropicalis and sample 48 and 49 and samples 58 and 59C. dublinensis (with sample 60). The discordant samples, as well as the samples in their clusters, were sequenced utilizing the ITS1 -5.8S-ITS2 region (Figure 3). The results confirmed rep-PCR clustering by identifying sample 47C. tropicalis and sample 58 and 59C. dublinensis.

**SUMMARY**

Rep-PCR methodology can be a useful tool for reliable identification of Candida species. This important factor for antibiotic resistance has been shown to vary by species. Therefore identification may have implications on patient treatment. In this case, C. tropicalis was actually one C. tropicalis and two C. dublinensis.

**REFERENCES**