

Abstract

DNA sequence analysis of the D2 region of the large subunit and the internal transcribed spacer 2 (ITS2) region of the ribosomal DNA have both been widely used to genotypically identify and classify molds and yeasts. A large-scale comparison of these two sequence targets was performed with fungal and yeast strains known to be relevant to pharmaceutical environmental monitoring programs, to determine which target was more powerful at distinguishing fungi at the species level. Sequence data was obtained by sequencing fungal species from culture collections as well as from downloading sequences from GenBank. The ability of each target to differentiate fungal species was evaluated by calculating the percentage of nucleotide differences between each species and its nearest neighbor. On average, the ITS2 region was observed to have a higher degree of variability and therefore higher differentiating power than the D2 region. *Komagataella* species were difficult to separate with the D2 region with only one base separating three species. In contrast, ITS2 sequencing could easily separate the three *Komagataella* species. Intra-species variation of the two targets was also compared to facilitate discussion of data interpretation.

Introduction

The identification of fungi is frequently based on microscopic and phenotypic criteria. These methods are very subjective and often can only taxonomically classify an organism to the Genus level or higher. With genotypic methods of identifying microorganisms becoming more commonplace, DNA sequencing is the clear choice for rapid and accurate identifications for yeasts and molds. There are two sequencing targets in the ribosomal RNA operon that have been commonly used for identification of fungal unknowns. The D1/D2 region of the large ribosomal subunit (LSU) and the internal transcribed spacer regions (ITS1/ITS2) (Figure 1). The D1-D2 expansion region of the LSU has traditionally been the target of choice for yeast identification, while the ITS region is more commonly used for filamentous fungi taxonomy. The ITS region has recently been recommended as a marker for fungal bar coding (Seifret, 2009). Differences in a specific region of ITS2, has also been shown to correlate with species delineation (Müller *et al.*, 2007). In the interest of simplifying and expediting fungal identification, shorter regions of these targets, D2 region (~350bp) and the ITS2 region (~350-450bp) have been selected as targets for rapid fungal identification (Accugenix, Inc.). A comparison of these two targets, their variability and their ability to differentiate species follows. Matched ITS2 and D2 sequence data sets were obtained from each of 236 strains by downloading sequences from GenBank or by direct sequencing of culture collection strains (Accugenix, Inc.). The strains represent 236 species commonly isolated from the environment, representing 98 different genera.

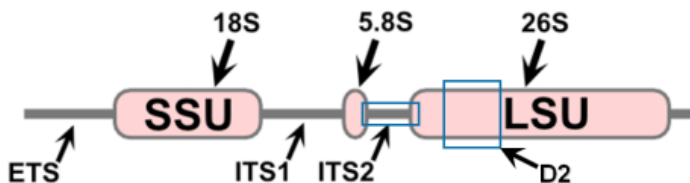


Figure 1. Diagram of the Fungal ribosomal RNA operon. ITS2 and D2 targets highlighted in blue squares.

Results

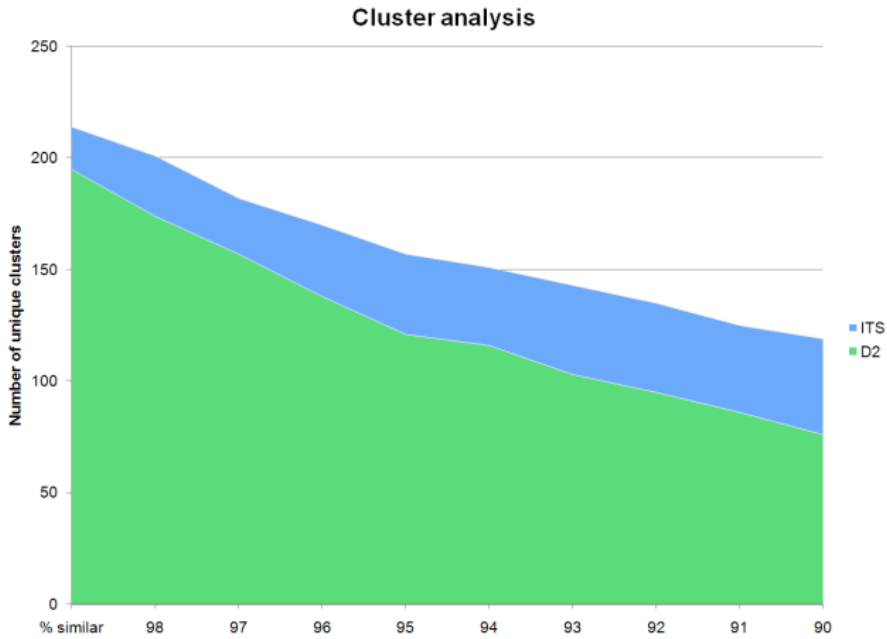


Figure 2. Graph comparing the number of unique sequence clusters at specific percent similarity cut-offs of D2 and ITS2 targets. Clusters were determined using the DOTUR furthest neighbor method based on a distance matrix calculated using ARB. (Schloss et al. 2005, Ludwig et al. 2004)

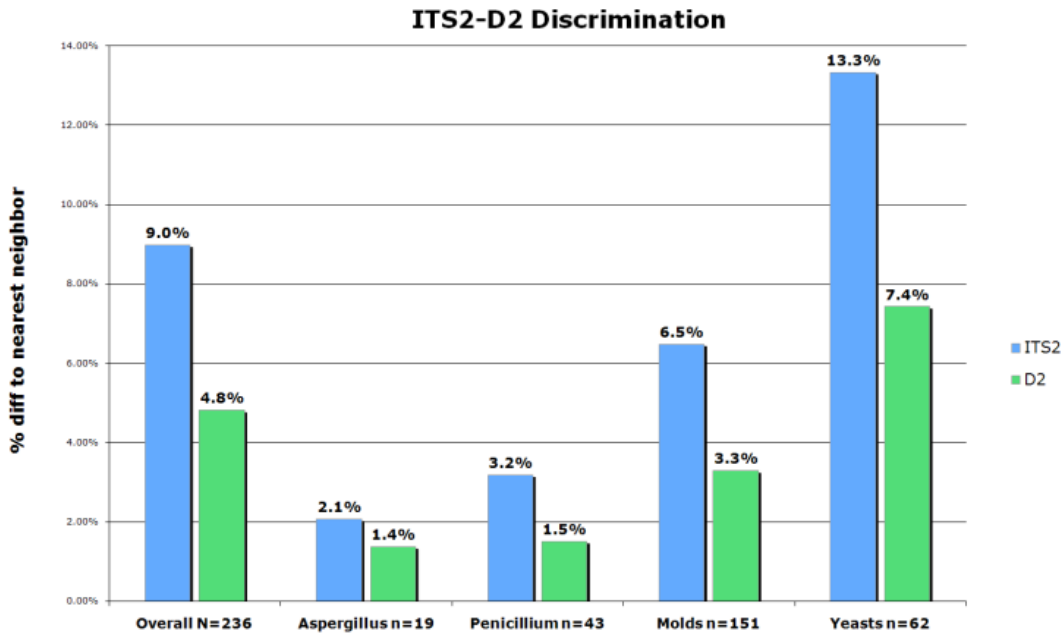


Figure 3. Graph summarizing the discriminatory power of the D2 and ITS2 targets. Discriminatory power was calculated based on the percent difference of each organism in the data set and its nearest neighbor using a pairwise alignment.

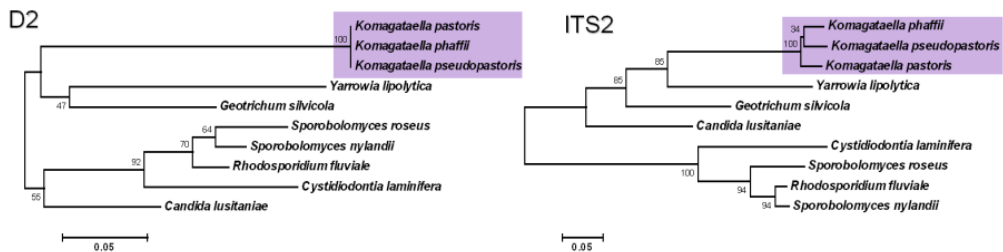


Figure 4. Phylogenetic trees comparing speciation of *Komagataella* sp. using D2 (left) and ITS2 (right) targets. Neighbor joining tree created using MEGA4 (500 bootstrap replicates) (Tamura et al. 2007)

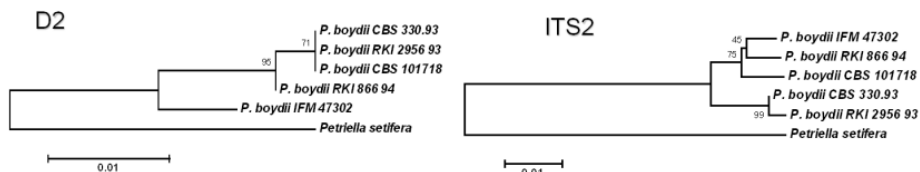


Figure 5. Phylogenetic trees depicting variation of different strains of *Pseudallescheria boydii* using D2 (left) and ITS2 (right) targets. Neighbor joining tree created using MEGA4 (500 bootstrap replicates) (Tamura et al. 2007)

Discussion

The cluster analyses and the species discrimination data (Figures 2 and 3) clearly show the ITS2 target has on average a higher degree of variation than the D2 region. This is likely due to the ITS2 region's location between two functional ribosomal subunits, whereas the D2 region is located within the LSU, likely causing it to be under more evolutionary constraint when compared to the ITS2 region. This higher degree of variability likely correlates to better species resolution as indicated in Figure 4 where the three *Komagataella* species cannot be differentiated with the D2 region but are clearly separated by the ITS2 target. The higher resolution is not limited to species distinction, as seen in Figure 5, where individual strains of *Pseudallescheria boydii* can be easily separated. This example of a highly variable species corroborates the recent data by Nilsson *et al.* (2008) which indicates that using standardized percentages for species "cut-offs" may not be applicable to the ITS target. In conclusion, the ITS2 target is more variable and has higher species resolution than the D2 region of the ribosomal RNA operon when used to identify environmental fungal isolates.

References

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About Us

Accugenix, Inc. provides leading-edge technology in microbial identification and characterization services. Our FDA-registered lab is cGMP compliant and maintains rigorous standards competitive at the global level. We specialize in testing, analyzing and interpreting data from environmental isolates commonly found in pharmaceutical, biotechnology, medical device, nutraceutical, personal care and other manufacturing industries.

For more than 20 years, Accugenix has provided the fastest, most accurate and reliable microbial identification services to over 400 facilities around the world. Accugenix updates its validated, proprietary DNA sequence libraries annually to reflect current taxonomy and newly described relevant species. We have the industry's first Fungal Library based on the ITS region. Since inception, we have tested more than 400,000 microorganisms – more than any other service laboratory in the industry, while maintaining an on-time delivery of over 99%.

History of Accugenix

1990.

Accugenix, Inc. began as Acculab, Inc., a reference laboratory specializing in microbial identification for industry and research clients. At the time we were one of only a few service laboratories in the world offering cellular fatty acid analysis, beginning a tradition of bringing cutting-edge microbiology methods to full commercial potential and utilization.

1999.

To reflect the addition of comparative DNA sequencing to our menu of validated methods, we created Accugenix, A Division of Acculab, Inc. Since then we have sequenced hundreds of thousands of environmental isolates from over 1000 pharmaceutical and biotechnology production facilities around the world, allowing us to build the largest and most unique industry database for bacteria and fungi that often occur in clean room manufacturing environments.

2005.

Our official name changed to Accugenix, Inc. on February 25, 2005.

2008.

Accugenix GmbH, our European subsidiary, was launched in Spring 2008.

Today.

Dedicated to being the industry leader for providing the most progressive microbiology methods available, Accugenix has invested in the technology, instrumentation and expertise to conquer genetic-based testing methods, their process validation, cGMP compliance, and other rigorous regulatory standards at the global level. Accugenix continues to staff its ranks with scientists and experts to guide and/or fast-forward your transition to genotypic microbial identification.



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