Is NMR spectroscopy a suitable tool in clinical microbiology?

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INTRODUCTION

Rapid and unequivocal identification of microbial pathogens is essential for efficient management of human infections. The identification of pathogenic microorganisms based on biochemical and assimilation tests is time consuming and often inaccurate. Therefore, molecular biological tests are increasingly used for reliable identification to the species level. This is in particular relevant for clinical and environmental yeast isolates, where phenotypic properties are sparse [1]. The ever-increasing number of potentially pathogenic yeasts has lead to a situation where the traditionally used criteria are not always sufficient for classification. While molecular techniques are precise, they are still laborious and expensive. NMR spectroscopy has proven to be a rapid and accurate alternative [2]. The aim of this study was twofold: (I) to evaluate a hierarchical statistical classification strategy for a broad and extendable NMR-based identification and (II) to use distance-based analysis methods for comparison of unknown yeast isolates.

METHODS

Microorganisms: A total of 1274 clinical environmental yeast isolates were cultured on Sabouraud dextrose agar plates for 24 or 48 hours at 30°C. These yeasts included the species *Candida albicans, C. dubliniensis, C. glabrata, C. parapsilosis, C. tropicalis, Clavispora lusitaniae, Cryptococcus neoformans, C. gattii, C. laurentius, C. humicolus, Issatchenkia orientalis, Pichia guillerimondii, Yarrowia lipolytica* and *Saccharomyces cerevisiae*. Comparison of environmental strains by distance-based methods was performed on 57 environmental *Metschnikowia* isolates. All yeasts were identified biochemically (VITEK YBC, API ID32 or ALLEV [3]) as well as by molecular methods (PCR fingerprinting, rDNA sequencing [1]).

NMR Spectroscopy: Colonies were suspended in 0.5 ml PBS/ D_2O containing 10^7 to 10^9 cells ml^{-1}. ^1H NMR spectra were obtained at 37°C on Bruker Avance 360 or 500 MHz NMR spectrometers using a 5-mm {^1H, ^{13}C} inverse-detection dual-frequency probe. ^1H NMR spectra were acquired with acquisition parameters as follows: pulse angle 90° (6-7 μs), repetition time 2.3s, 4k data points, 32 transients, spectral width 10 ppm, water suppression by selective excitation field gradients.

Classification of NMR spectra: A hierarchical identification system for clinical isolates was developed based on pair-wise classifiers [2, 4] that considered the taxonomic levels shown in Fig.1. This system was tested against pair-wise classifiers comparing all possible species combination seen in Fig.1.

Distance-based classification was performed for the *Metschnikowia* species using BioloMICS (BioAware).

Results and Discussion

In the case of a large number of classes (species), pair-wise classification requires the development of an unfeasibly large number of classifiers (55 in the case of the 11 yeasts). Similar accuracies were achieved when the conventional pair-wise classification (97% agreement with molecular identification) was compared with the hierarchical classification shown in Fig.1 (13 pair-wise classifiers, 95% agreement with molecular identification). The hierarchical system was also tested against species not included in the test set, but belonging to one of the "higher" taxonomic levels. In 90% of these cases, the respective isolates were assigned to the correct taxonomic class, proving that the hierarchical approach can easily be extended for new emerging species.

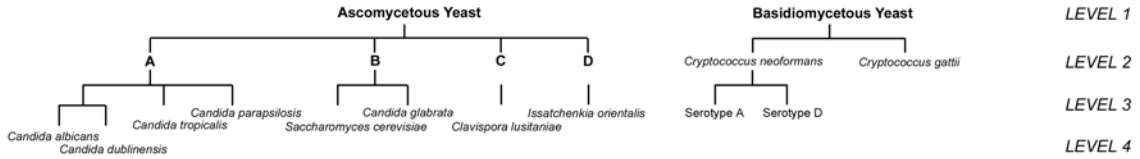

Fig.1: Hierarchical classification of the pathogenic yeast species utilized for the training set.

NMR spectra of physiologically identical isolates of the genus *Metschnikowia* were compared visually and by development of a similarity matrix. Two well-separated clusters indicated the existence of distinct taxa, which were later confirmed by molecular tests, indicating the value of NMR spectroscopy for rapid screening in microbiology.

CONCLUSION

Statistical classification of NMR spectra is a suitable technique for rapid, robust and potentially automated identification of pathogenic yeasts in clinical microbiology laboratories.

REFERENCES

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