Species and sub-species identification of Candida clinical isolates by FTIR and RAPD techniques

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Fourier Transform Infrared (FTIR) spectroscopy is a "whole-cell fingerprinting" method based on the interaction between light and matter. It has been proven to be useful in the macromolecular characterisation of various biological samples such as tissues, cells, bacteria and fungi. It has been applied to the identification of microorganisms at the genus and species level.

In one of our previous studies on *Candida* clinical strains, we have previously shown that FTIR can not only discriminate between *Candida* species, but also has the potential, under controlled experimental conditions, to identify sub-species of *C. albicans*. These findings have led us to develop FTIR spectroscopy a new phenotypic approach to type *C. albicans* clinical isolates.

Candida albicans is an opportunistic pathogen, generally causing infections of endogenous origin. Nevertheless, several outbreaks of candidiasis have been described. Molecular typing methods (RAPD, PFGE, Ca3 fingerprinting …) are generally used to determine the genetic relationship between the infectious strains. However, there is no consensus about the most efficient method. Association of several methods is generally recommended in order to enhance the discriminative power.

This work reports on two case studies where FTIR spectroscopy has been tested in real clinical situations. The first one concerns an epidemiological survey of ICU patients for cross-infections, where seventy-nine strains, isolated from multiple anatomical sites of 9 ICU adult patients over a period of 4 months, were analysed. The second study investigates an oropharyngeal candidiasis outbreak in a maternity unit where sixteen neonates were infected by *C. albicans* in the first 10-days of life and this over a 4-month period. Only eight strains isolated from oropharyngeal swabs from 8 neonates were available for the typing study. No yeast was isolated from the hands of the staff or from potential environmental reservoirs (milk, feeding-bottle, tongue-depressor …). FTIR spectra were recorded in transmission mode on thin transparent dried fungal films. Spectra reflect the total biochemical composition of the studied sample. The spectral signatures being too complex to be analysed by visual inspection, statistical analysis methods such as hierarchical cluster analysis (HCA), principal component analysis (PCA) and discriminant analysis (DA) were to be used to compare the spectral signatures. All strains were also identified in parallel by a clinical laboratory reference genotypic technique based on the RAPD technique (Randomly Amplified Polymorphism DNA).

In the case of the adult ICU patients, FTIR spectroscopic classification reveals 9 distinct groups corresponding to each patient. All strains (multiple anatomical sites) belonging to the same patient could also be grouped together. This result revealed that no cross-contamination occurred in this ICU unit. This was in complete concordance with the RAPD molecular typing.

Data obtained on the eight neonate *C. albicans* strains were compared to 2 adult *C. albicans* strains, used here as reference both in FTIR spectroscopy and RAPD. FTIR spectra were classified using HCA and results showed three distinct clusters. The 2 adult strains used as reference were easily differentiated from the neonate strains and formed two separate clusters. The spectra of the 8 neonate strains were all grouped in the third cluster and could not be differentiated. We also found that the 8 neonate strains presented the same banding pattern with the 2 RAPD primers used, whereas the 2 adult strains presented different patterns. These concordant results suggest that the neonate strain population had a clonal origin and that horizontal transmission occurred. However, the origin of the infection could not be elucidated before the outbreak stopped.

This study is the first to demonstrate that a nosocomial transmission of *C. albicans* can be detected by FTIR spectroscopy. This novel approach constitutes a rapid and simple way for typing strains in less than 2 hours starting from a pure culture. We believe that FTIR-based strain typing can well be extended to other clinically relevant *Candida* species such as *C. glabrata* and *C. parapsilosis*. This technique can be proposed as a first step during epidemiological investigations, followed by a genotyping method. Such a procedure could contribute to accelerate the implementation of control measures in a clinical setting.