Matrix-assisted laser desorption/ionization time-of-flight intact cell mass spectrometry to detect emerging pathogenic Candida species

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Abstract

Matrix-assisted laser desorption/ionization time-of-flight intact cell mass spectrometry (MALDI-TOF-ICMS) was used to differentiate pathogenic Candida species, difficult to identify by traditional methods such as growth and biochemical reactions. Results showed that species complexes like C. parapsilosis, C. orthopsilosis, and C. metapsilosis, and very closely related species like C. glabrata and C. dubliniensis, could be clearly separated. MALDI-TOF-ICMS stands out as a promising tool for the rapid detection of emerging pathogens.

Keywords: Candida; C. parapsilosis complex; MALDI-TOF ICMS

The clinical impact of fungal infections has greatly increased in the last years, particularly in immunocompromised hosts. Yeasts belonging to the genus Candida have emerged as the major opportunistic pathogens in these patients and currently constitute the fourth most common cause of nosocomial infections in intensive care units (Perlroth et al., 2007; Rueping et al., 2009). Candida albicans is the species most commonly isolated, although others, such as C. tropicalis, C. krusei, C. parapsilosis, and C. glabrata, have increasingly been recognized as pathogens with a wide distribution (Pfaller and Diekema 2007, Perlroth et al., 2007). Additionally, new species have been described, for example, C. dubliniensis related to C. albicans (Sullivan et al., 1995); C. orthopsilosis and C. metapsilosis, very closely related to C. parapsilosis (Tavanti et al., 2005); and C. bracarensis, phenotypically similar to C. glabrata (Correia et al., 2006), which are emerging as potential pathogens.

Early identification of the species causing infection is crucial for adequate treatment since the antifungal susceptibility profile differs greatly among species. Moreover, the time needed for the identification of the pathogen is an important determinant of infection-related mortality rates in hospitalised patients. The methods currently used for yeast identification in clinical laboratories are not able to distinguish emerging pathogenic species and, additionally, are either time consuming and/or require considerable expertise.

Several reports have shown that matrix-assisted laser desorption/ionisation time-of-flight intact cell mass spectrometry (MALDI-TOF ICMS) is suitable for the fast and reliable identification of pathogenic microorganisms (Barbuddhe et al., 2008; Claydon et al., 1996; Degand et al 2008; Mellman et al., 2008), including filamentous fungi (Santos et al., 2010) and yeasts (Marklein et al., 2009; Qian et al., 2008; Putignani et al., 2011; Stevenson et al., 2010; van Veen et al., 2010). The performance and cost analysis of MALDI-TOF ICMS for routine identification of yeast were recently evaluated by Dhiman et al. (2011) who concluded that this is an accurate, rapid, and cost-effective technique that could be applied in a medical microbiology laboratory for identification of yeast clinical isolates. Therefore, the aim of the present work was to use MALDI-TO-ICMS for the identification of Candida sp.
Sixty-seven clinical isolates, belonging to 9 Candida species, namely, C. albicans, C. tropicalis, C. kruzei, C. parapsilosis, C. orthopsilosis, C. metapsilosis, C. lusitaniae, C. glabrata, and C. bracarensis, were analysed. Two strains of Lodderomyces elongisporus and 4 Saccharomyces cerevisiae isolates were also included in the study. Candida clinical isolates were obtained from several hospitals and health institutions and collected from blood, bronchial secretions, urine, and vaginal exudates. All isolates were identified by identification cards (BioMerieux, France) at the institutions of origin, and most were confirmed by 26S rDNA and ITS sequencing at the Department of Biology, University of Minho. The isolates were cryopreserved at −80 °C. Yeast cells were grown on YEPD agar medium plates for 24 h, at 37 °C. A loopful of yeast cells was directly transferred from the culture medium onto each position of the 48-well flex target plate, and 0.5 μL of 25% formic acid was immediately mixed with the yeast. After evaporation, 0.5 μL matrix solution (75 mg/mL 2,5-dihydroxybenzoic acid in ethanol/water/acetonitrile [1:1:1] with 0.03% trifluoroacetic acid) was added and gently mixed. All sample mixtures were air dried at room temperature. Each isolate was spotted in triplicate.

Analyses were performed on an Axima LNR system (Kratos Analytical, Shimadzu, Manchester, UK) equipped with a nitrogen laser (337 nm). The mass range from 2,000 to 20,000 Da was recorded by using the linear mode. Escherichia coli ribosomal proteins were used for external calibration of the spectra. Spectra were exported to the SARAMIS™ software package (Spectral Archiving and Microbial Identification System, AnagnosTec, Postdam-Golm, Germany, http://www.anagnostec.eu) where the final identifications were achieved. Cluster analysis of the MALDI-TOF ICMS mass spectral data was performed using the SARAMIS™ database by comparing database peak lists of individual samples with SuperSpectra and/or reference spectra. The mass spectra obtained presented peaks ranging from 2,000 to 12,000 Da, with the most intense within the range of 5,000 to 7,000 Da. Mass spectra of the different Candida species analyzed showed distinctive features that could be used for species differentiation, and examples of distinctive spectra are shown in Fig. 1.

Initially, the isolates of C. metapsilosis, C. orthopsilosis, and C. bracarensis could not be identified since spectra for appropriate reference strains were not available in the database. As the identity of the isolates analysed in this study had been previously confirmed by D1/D2 26S rDNA and ITS sequencing, a reference spectrum for each was introduced into the database and subsequent comparison with the unidentified species resulted in a matching profile for all the isolates.

The species presenting the highest number of peaks were the C. glabrata and C. bracarensis isolates, which both showed an additional intense peak at around 11,000 Da. C. parapsilosis and C. metapsilosis isolates were the ones yielding a lower number of peaks. Additionally, the closely related species, C. parapsilosis, C. metapsilosis, and C. orthopsilosis, and C. glabrata and C. bracarensis, presented similar but yet distinguishable profiles (Fig. 1).

L. elongisporus, a species hypothesized to be C. parapsilosis teleomorph (James et al., 1994) and considered very closely related by sequence analysis (Butler et al., 2009), presented a completely distinct profile (Fig. 1). This observation is particularly interesting since L. elongisporus has been recently described in cases of bloodstream infection misidentified as C. parapsilosis (Lockhart et al., 2008a).

C. glabrata and C. bracarensis presented similar spectra. The S. cerevisiae profile was distinctive, although peaks in the same spectral region as for C. glabrata and C. bracarensis (between 4,000 and 6,600 Da) could be observed. These data confirm the close genomic and proteomic relation between S. cerevisiae and C. glabrata, as described in the literature (Roetzer et al., 2011), but still the 3 species appear as distinct.

Cluster analysis of the MALDI-TOF ICMS mass spectral data included all peaks obtained from 2,000 to 20,000 Da.

![Fig. 1. Examples of representative MALDI-TOF ICMS spectra of the yeast species analysed in this study.](image-url)
Mass spectral signatures allowed the grouping of all isolates into clusters according to their species designation (Fig. 2). These results are in accordance with the ones reported previously (Bader et al., 2010; Marklein et al., 2009; Qian et al., 2008; Stevenson et al., 2010). At a threshold of about 48% similarity, all different species were clearly distinguished except the pairs \( C. \text{parapsilosis}/C. \text{orthopsilosis} \) and \( C. \text{albicans}/C. \text{dubliniensis} \), which could only be distinguished at a threshold of 57% and 52%, respectively. \( C. \text{dubliniensis} \) isolates were not analysed in the present work but spectra, obtained from the SARAMIS database, were included in the analysis. Spectra obtained for all isolates belonging to the same species group together, presenting a very close profile.

All of the closely related species, \( C. \text{albicans} \) and \( C. \text{dubliniensis} \); \( C. \text{parapsilosis} \), \( C. \text{metapsilosis} \), and \( C. \text{orthopsilosis} \); as well as \( C. \text{glabrata} \) and \( C. \text{bracarensis} \), could be clearly distinguished. Also, \( S. \text{cerevisiae} \) and \( L. \text{elongisporus} \) species were distinctly separated. Due to their close phylogenetic relationship with the species most commonly found, the new species mentioned above are often misidentified and MALDI-TOF ICMS analysis can be a powerful high-throughput proteomic technique to discriminate closely related \( Candida \) species, which is feasible otherwise only by molecular biology techniques. The equipment is straightforward to operate and inexpensive on an individual sample basis. The rapid identification of uncommon yeast species that are emerging as human pathogens may be accurately achieved provided that a complete and quality-controlled database is available.

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References


