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Discrimination of *Candida* species by paper spray mass spectrometry

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This work is dedicated to Veronica Bierbaum in recognition of the precision and range of her science and her leadership in the mass spectrometry community.

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ABSTRACT

Ambient ionization mass spectrometry using paper spray is utilized for rapid discrimination of *Candida* species without sample preparation. The experiment is based on characteristic lipid profiles and is an extension to a procedure used for the discrimination of bacteria. *Candida* colonies were smeared onto filter paper pre-cut to a sharp tip, subsequently wetted with solvent and held at high potential. Charged droplets released by field emission were sucked into the mass spectrometer inlet and mass spectra were recorded. Eight closely related *Candida* species with clinical relevance and known phylogeny were investigated. Numerical data fusion of the positive and negative ion mass spectra and multivariate statistics (principal component analysis, followed by linear discriminant analysis) allowed species level discrimination with a prediction rate of ca. 90%.

1. Introduction

Candida is one of the most common etiological agents of nosocomial bloodstream infections (candidemia) which are a major cause of in-hospital morbidity and mortality [1–4]. The prevalence of candidemia has been reported to be approximately 6.9 per 1000 patients [2,5]. Invasive candidemia is life-threatening with a high mortality rate (30–40%) which can be decreased by appropriate and timely antibiotic therapy [1,2,5–11]. Thus, prompt identification of candidemia is desired but not currently successful, because clinical symptoms are aspecific and the standard technique used to confirm suspected cases is blood culture, which lacks of analytical sensitivity (approximately 50%) [2,11–13].

Candidemia is primarily caused by *Candida albicans* but non-*albicans* species are increasingly common and clinically relevant [1–4,8,15,19–21]. There are more than 20 known *Candida* species, each with different antibiotic susceptibility profiles [1,15–17,1,20,22,23]; therefore, effective treatment depends on the pathogen [14,18,20]. Hence a simple, rapid, cost-effective, and accurate method that allows for species discrimination is needed [24].

Yeasts, such as *Candida*, can be characterized using morphological, physiological, and biochemical criteria [24–27], but these are generally time consuming and often yield ambiguous results [1,2,12,28–30]. Newer methods used to quickly speciate *Candida* take advantage of species-specific enzymatic characteristics [27,31,32]. Various molecular techniques, including real-time PCR [23,33–37], karyotypes by pulsed field gel electrophoresis [25,38], fluorescent *in situ* hybridization [39,40], and pyrosequencing [41], have been developed to speed the identification of bloodborne yeasts.

Mass spectrometry, increasingly used for microorganism identification, allows biomolecule detection from complex matrices and recovery of characteristic profiles (*e.g.*, lipids). Limiting the discussion to direct MS analysis (*i.e.*, without coupling to chromatography), MALDI of protein extracts has been shown to be a powerful biotyping tool for bacteria [29,42–46], while less extensively applied to yeast [47–50]. In general, characteristic protein signals are harder to obtain for yeast than for bacteria [51,52], but with optimization, specific profiles can be obtained [50,53]. Paper spray mass spectrometry (PS-MS) has been used for analysis of complex mixtures under ambient conditions [27–29]. PS-MS has been recently adopted to detect characteristic lipid profiles of closely related bacteria [54].

This study presents the application of PS-MS for the rapid discrimination of *Candida* samples *in vitro* in less than 2 min. Eight *Candida* species have been selected and analyzed by a bipolar

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PS-MS experiment (in which positive and negative ion mode mass spectra were collected in a single step from the same sample). The species chosen account for as much as 95–98% of all the bloodborne yeasts [1]. Due to the large amount of information acquired in full scan mass spectra, multivariate statistics is necessary to manage the data and extract relevant chemical features. In this study, principal component analysis (PCA) was used to explore the differences among *Candida* species. Then taking advantage of the PCA, the generation of which acts as a data reduction technique, linear discriminant analysis (LDA) was applied as a supervised classification method [55]. The positive and negative MS data were merged *via* mid-level data fusion in order to improve overall prediction of the *Candida* spp. investigated resulting in a prediction rate of ~90% [54,56,57].

2. Experimental

2.1. Chemicals and materials

Methanol (HPLC grade) was purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ). *N*,*N*-Dimethylformamide (DMF), chloroform and acetonitrile were purchased from Sigma–Aldrich (St. Louis, MO). The culturing medium, Sabouraud agar (SAB), was purchased from Remel (Lenexa, KS). Sterile inoculation loops were purchased

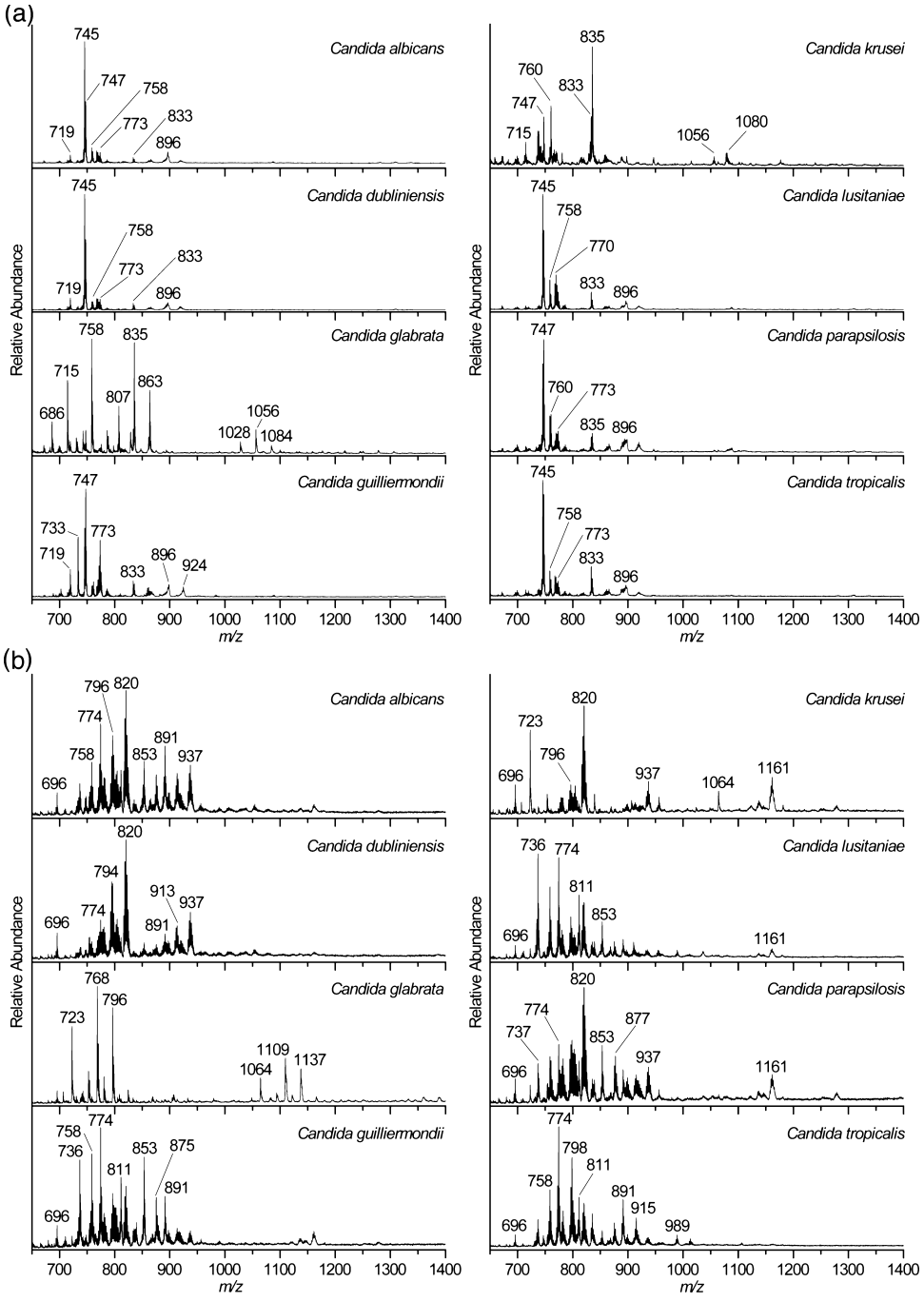

Fig. 1. (a) Mass spectra (average of all replicates per species, normalized by the total ion current) obtained by PS-MS of eight different *Candida* species in the negative ion mode. (b) Average and normalized mass spectra obtained by positive mode PS-MS.