Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry

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Abstract

The key to therapeutic success with yeast infections is an early onset of antifungal treatment with an appropriate drug regimen. To do this, yeast species identification is necessary, but conventional biochemical and morphological approaches are time-consuming. The recent arrival of biophysical methods, such as matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), in routine diagnostic laboratories holds the promise of significantly speeding up this process. In this study, two commercially available MALDI-TOF MS species identification systems were evaluated for application in clinical diagnostics, using a geographically diverse collection of 1192 clinical yeast and yeast-like isolates. The results were compared with those of the classical differentiation scheme based on microscopic and biochemical characteristics. For 95.1% of the isolates, all three procedures consistently gave the correct species identification, but the rate of misclassification was greatly reduced in both MALDI-TOF MS systems. Furthermore, several closely related species (e.g. *Candida orth*opsilosis/*metapsilosis*/*parapsilosis* or *Candida glabrata*/*bracarensis*) could be resolved by both MALDI-TOF MS systems, but not by the biochemical approach. A significant advantage of MALDI-TOF MS over biochemistry in the recognition of isolates novel to the system was observed. Although both MALDI-TOF MS systems employed different approaches in the database structure and showed different susceptibilities to errors in database entries, these were negligible in terms of clinical usefulness. The time-saving benefit of MALDI-TOF MS over biochemical identification will substantially improve fungal diagnostics and patient treatment.

Keywords: Biotyper 2.0, human pathogenic fungi, MALDI-TOF MS, Saramis, yeast identification
Original Submission: 1 July 2010; **Revised Submission:** 30 September 2010; **Accepted:** 30 September 2010
Editor: G. Greub
Article published online: 14 October 2010
Clin Microbiol Infect 2011; **17:** 1359–1365
10.1111/j.1469-0691.2010.03398.x

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Introduction

Yeast infections are a significant cause of morbidity and mortality in critically ill patients, e.g. those undergoing immunosuppressive therapy, those recovering from surgery, or in those infected by human imunodeficiency virus. Early therapeutic intervention is critical for successful treatment of yeast infections [1–3], and the optimal choice of antifungal drugs will ultimately be based on two key factors: (i) the fungal species and its intrinsic resistance; and (ii) the result of the *in vitro* resistance testing of the individual isolate [4].

The *Atlas of Clinical Fungi* lists approximately 400 fungal species with clinical relevance and human pathogenic potency [5], causing a wide range of clinical symptoms, ranging from local inflammation to life-threatening disseminated disease. Unfortunately, conventional laboratory differentiation of yeasts, involving, for example, microscopy and biochemical tests, not only requires up to several days, but is also cost-intensive and requires extensively trained laboratory personnel. At present, in those cases where classical methods give unclear results, the isolates need to be re-analysed by sequencing of species-specific regions. This is especially true for rare, potentially emerging yeast pathogens that are not identifiable by standard tests. Faster

TABLE 1. Species distribution within the test set

Species	No. of isolates	Biochemical	Biotyper	Saramis
Blastoschizomyces capitatus (*Geotrichum capitatum*)	3	Y	N	N
Candida albicans	512	Y	Y	Y
Candida bracarensis	1	N	N	N
Candida dubliniensis	8	Y	Y	Y
Candida glabrata	272	Y	Y	Y
Candida metapsilosis	1	N	Y	N
Candida orthopsilosis	8	N	Y	N
Candida parapsilosis	105	Y	Y	Y
Candida pararugosa	3	N	N	N
Candida rugosa	8	Y	Y	N
Candida tropicalis	88	Y	Y	Y
Candida viswanatii	1	N	N	N
Clavispora lusitaniae (*Candida lusitaniae*)	14	Y	Y	Y
Cryptococcus neoformans	7	Y	Y	Y
Galactomyces geotrichum (*Geotrichum candidum*)	1	N[a]	N	Y
Geotrichum clavatum	3	N[a]	N	N
Issatchenkia orientalis (*Candida krusei*)	53	Y	Y	Y
Kluyveromyces marxianus (*Candida kefyr*)	21	Y	Y	Y
Kodamaea ohmeri (*Candida guilliermondii* var. *membranaefaciens*)	2	Y	Y	N
Pichia anomala (*Candida pelliculosa*)	3	Y	Y	Y
Pichia cactophila (*Candida inconspicua*)	7	Y	Y	Y
Pichia fabianii (*Candida fabianii*)	7	N	N	N
Pichia farinosa (*Candida cacaoi*)	2	Y	Y	Y
Pichia fermentans (*Candida lambica*)	1	Y	Y	Y
Pichia guilliermondii (*C. guilliermondii* var. *guilliermondii*)	23	Y	Y	Y
Pichia jadinii (*Candida utilis*)	1	Y	Y	Y
Pichia membranifaciens (*Candida valida*)	1	Y	Y	Y
Pichia norvegica (*Candida norvegensis*)	5	Y	Y	Y
Rhodotorula mucilaginosa	2	Y	Y	Y
Saccharomyces cerevisiae	20	Y	Y	Y
Trichosporon asahii	4	Y	Y	Y
Yarrowia lipolytica (*Candida lipolytica*)	1	Y	Y	Y
Uncharacterized species	4	N	N	N

Y, represented in the database; N, not represented in the database.
A total of 1192 clinical yeast isolates across the fungal phylum representing 32 known and four uncharacterized species were represented in the test set. Teleomorph–anamorph relationships and conspecific species, as described by deHoog [5] and others [29,30], necessary to resolve ambiguous nomenclature between the databases used in this study are given in parentheses.
[a]Genus level only.

species identification, with rapid determination of the particular species-specific intrinsic resistance, would be an important step forwards in the successful management of life-threatening fungal diseases.

Recently, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been successfully introduced for rapid species identification of microorganisms in the clinical laboratory. With this method, crude cell extracts can be used to identify the species of a given isolate by comparison of the mass patterns within approximately 2–20 kDa with a database containing the patterns of reference strains. These patterns are highly species-specific [6–8], and may even allow subspecies identification [9]. As this process takes only minutes, the introduction of MALDI-TOF MS into diagnostic laboratories holds the promise of significantly speeding up diagnostic processes while simultaneously leading to more accurate identification of pathogens [10,11]. Experimental data also indicate that MALDI-TOF MS can increase the resolution at which fungal species, such as different moulds [12–15] and yeasts [9,16], can be differentiated from each other. Therefore, in the clinical laboratory, MALDI-TOF MS-based differentiation could substantially improve the quality of and reduce the time needed for the identification of yeasts.

To evaluate the clinical use of the two currently commercially available MALDI-TOF MS systems (MALDI BioTyper2 (Bruker Daltonics, Bremen, Germany) and Saramis (AnagnosTec, Potsdam, Germany)) in yeast identification, a collection of 1192 clinically relevant yeast and yeast-like isolates was established (Table 1), reflecting a species distribution as it is encountered during clinical routine. Both mass spectrometry systems were compared with each other and with the classical approach for fungal species identification in diagnostic laboratories.

Materials and Methods

Cultivation of fungi

Yeasts were kept either as cryobank stocks (Mast Diagnostica, Reinfeld, Germany) or as snap-frozen liquid YPD-glycerol stocks (1% yeast extract, 2% peptone, 1% glucose, 25%