JCM
Journals.ASM.org

Optimization of the Preanalytical Steps of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Identification Provides a Flexible and Efficient Tool for Identification of Clinical Yeast Isolates in Medical Laboratories

Marianne Goyer,[a] Geraldine Lucchi,[b] Patrick Ducoroy,[b] Odile Vagner,[a] Alain Bonnin,[a] and Frederic Dalle[a]

Mycology & Parasitology Department, University Hospital, Dijon, France,[a] and CLIPP (Clinical Innovation Proteomic Platform), IFR 100 Santé STIC, University of Burgundy and University Hospital, Dijon, France[b]

We report here that modifications of the preanalytical steps of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identification of yeasts, with regard to the original protocol provided by the manufacturers, appear to be efficient for the reliable routine identification of clinical yeast isolates in medical laboratories. Indeed, when one colony was sampled instead of five and the protein extraction protocol was modified, the performance of MALDI-TOF MS was superior to that of the API ID 32C method (discrepancies were confirmed by using molecular identification), allowing the correct identification of 94% of the 335 clinical isolates prospectively tested. We then demonstrated that the time for which the primary cultures were preincubated on CHROMagar did not impact the identification of yeasts by MALDI-TOF MS, since 95.1 and 96.2% of the 183 clinical yeast isolates prospectively tested were correctly identified after 48 and 72 h of preincubation, respectively.

In most clinical microbiology laboratories, medically important yeasts are routinely identified by the use of chromogenic agar medium and/or enzymatic or biochemical tests (8, 11). However, these conventional methods are time-consuming, with identification times ranging from 48 to 96 h after yeast recovery from clinical specimens. Recently, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) technology has been suggested to be suitable for the fast and reliable identification of clinical yeasts and yeast-like isolates (1, 3–6, 9, 10), including rare species of yeasts that are increasingly isolated in medical mycology and often associated with resistance to antifungal drugs (7).

The aim of our study was to optimize the preanalytical steps of MALDI-TOF MS identification of yeast isolates by evaluating (i) whether protein extraction from only a single colony could produce accurate identification of yeasts and (ii) whether incubation of the primary culture for 72 h instead of 48 h, as suggested by the manufacturer, would significantly impact the performance of the identification method. Indeed, these modifications would provide more flexibility in the routine identification of yeasts in medical laboratories.

To evaluate the efficiency and reliability of modifications of the preanalytical steps of MALDI-TOF MS identification of yeasts, this study was conducted in two phases: first, in order to evaluate modifications proposed for the protocol of protein extraction, 335 clinical isolates were prospectively collected from various clinical specimens received by the mycology laboratory of the University Hospital of Dijon during a 3-month period. These specimens were cultured on CHROMagar medium at 30°C for 48 h. For the second part of the study, comparing preincubation for 48 versus 72 h before MALDI-TOF MS, 183 new clinical isolates were prospectively collected under the same conditions during a 2-month period. Specimens were cultured on CHROMagar and incubated for 48 h at 30°C. Then, one colony was sampled for MALDI-TOF identification. CHROMagar was then incubated for an additional 24 h before a second identification by MALDI-TOF. Each isolate collected was tested in parallel by MALDI-TOF MS and a biochemical method (API ID 32C; bioMérieux, Marcy l'Etoile, France). The data obtained by the two methods were then compared, and for each isolate, identification was considered concordant when the same identification was obtained with both the MALDI-TOF MS and API ID 32C methods. In the case of discrepant identifications, isolates were subsequently identified by sequencing of the internal transcribed spacer 2 region of the rRNA gene as described by Chen et al. (2). MALDI-TOF MS analyses were performed after 48 and 72 h of primary culture with an UltraFlex II with the MALDI BioTyper 3.1.2. software (Bruker Daltonics). The Bruker Bacterial Test Standard was used for calibration of the mass spectrometer. For each spectrum, 1,000 shots were summed in linear and positive mode in a range of 2,000 to 18,000 Da. The identification scores were used as recommended by the manufacturer; a score of ≥ 2 means identification to the species level, and a score between 1.7 and 1.9 means identification to the genus level. Two successive scores between 1.7 and 1.9 corresponding to the same species allowed identification to the species level. Any other cases did not allow identification to the species level.

Recently, Marklein et al. (6) described the MALDI-TOF MS identification of yeasts by the sampling of five representative single colonies of the strain for the protein extraction step. In the present study, protein extraction was done by sampling a single

Received 14 March 2012 Returned for modification 10 April 2012
Accepted 11 June 2012
Published ahead of print 20 June 2012
Address correspondence to Frédéric Dalle, frederic.dalle@u-bourgogne.fr.

doi:10.1128/JCM.06381-11

TABLE 1 Identification of clinical yeast isolates by MALDI-TOF MS after 48 or 72 h of preincubation

Species or parameter	No. of isolates	No. correctly identified by MALDI-TOF MS after preincubation for:	
		48 h	72 h
Candida albicans	50	50	50
Candida glabrata	24	23	24
Candida kefyr	28	28	27
Candida krusei	15	15	15
Candida tropicalis	19	16	16
Candida lusitaniae	1	1	1
Candida parapsilosis	25	21	22
Candida norvegensis	2	2	2
Saccharomyces cerevisiae	15	15	15
Pichia guilliermondii	1	1	1
Arxiozyma telluris	1	1	1
Trichosporon mucoides	1	0	1
Debaryomyces hansenii	1	1	1
Total no. of isolates (% correctly identified)	183	174 (95.1)	176 (96.2)

TABLE 2 Details of discordant results of yeast identification by MALDI-TOF MS after 48 or 72 h of preincubation on CHROMagar

Molecular identification and strain no.	Identification after preincubation on CHROMagar at 30°C[a]	
	48 h	72 h
C. tropicalis		
1	*Filifactor villosum*	***C. tropicalis***
2	***C. tropicalis***	*Filifactor villosum*
3	*Filifactor villosum*	***C. tropicalis***
4	*Filifactor villosum*	***C. tropicalis***
5	***C. tropicalis***	Not reliable
6	***C. tropicalis***	Not reliable
C. parapsilosis		
7	***C. parapsilosis***	*Filifactor villosum*
8	*Filifactor villosum*	***C. parapsilosis***
9	Not reliable	***C. parapsilosis***
10	Not reliable	***C. parapsilosis***
11	***C. parapsilosis***	Not reliable
12	Not reliable	***C. parapsilosis***
13	***C. parapsilosis***	Not reliable
C. glabrata		
16	Not reliable	***C. glabrata***
17	***C. glabrata***	Not reliable
C. kefyr, 18	***C. kefyr***	*C. glabrata*
Trichosporon mucoides, 19	Not reliable	***Trichosporon mucoides***

[a] Correct identifications are in boldface.

representative colony. Consequently, the extraction protocol was modified as follows. After 48 h of incubation at 30°C on CHROMagar (BD) medium, a single representative colony was mixed thoroughly in 0.3 ml of double-distilled water. Then, 0.9 ml of absolute ethanol was added to the suspension. After mixing, the tubes were centrifuged at 20,000 × *g* for 2 min, the supernatant was discarded, and the pellet was air dried. The pellet was then mixed thoroughly with 20 μl of formic acid before an equivalent volume of acetonitrile was added. After centrifugation (20,000 × *g* for 2 min), 1 μl of the supernatant was placed onto a polished ground steel MALDI target plate and allowed to dry at room temperature. Each sample was overlaid with 2 μl of matrix (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid) and air dried at room temperature. A total of 335 clinical isolates were collected during a 3-month period (284 *Candida* species, 22 *Pichia* species, 14 *Geotrichum* species, 13 *Saccharomyces cerevisiae*, and 2 *Trichosporon* species) and prospectively processed for MS identification by using the new extraction protocol.

MALDI-TOF MS yielded correct identification to the species level of 315 (94%) of the 335 clinical isolates on the first attempt. *Candida famata*, *Candida lambica*, *Candida magnoliae*, *Trichosporon* species, and species of the genus *Geotrichum* (15 clinical isolates) were not identified because appropriate spectra were not included in the 3.1.2 version of the database. However, five clinical isolates originally misidentified were correctly identified after a second extraction. Only 80.5% of the clinical isolates prospectively tested were correctly identified by biochemical methods.

In the first part of this study, MALDI-TOF MS identifications were performed after sampling of colonies obtained in CHROMagar medium incubated for 48 h at 30°C as recommended by the manufacturer. In the second part, we evaluated the impact of a 72-h preincubation time on the quality of the spectra and identification with MALDI-TOF MS technology. A new set of 183 clinical isolates were prospectively tested within a 2-month period. For each CHROMagar plate, corresponding to individual biological samples, one colony of yeast was picked up and processed for MALDI-TOF MS identification after 48 h of preincubation at 30°C. In the case of mixed cultures containing different species of yeasts, as indicated by the presence of colonies of different colors upon the corresponding CHROMagar plate, one colony of each of the different species of yeast indicated was processed for MALDI-TOF MS identification. For each isolate tested after 48 h of preincubation, the corresponding CHROMagar plate was then incubated for an additional 24 h and then a new colony was processed for MALDI-TOF MS identification. In the case of mixed cultures, sampling of colonies was done as described above. Each protein extraction was done as described above. The results obtained after 48 and 72 h of preincubation before extraction were comparable, allowing 95.1 and 96.2% correct identification, respectively (Table 1). Of the 12 clinical isolates that were misidentified after 48 h of preincubation, 9 were correctly identified after 72 h of preincubation. Conversely, of the 11 clinical isolates misidentified after 72 h of preincubation, 8 were correctly identified after 48 h of preincubation (Table 2). For one isolate, MALDI TOF MS identification after 48 h gave *Candida kefyr* and *Candida glabrata* after 72 h whereas the molecular identification result was *C. kefyr* (2). Consequently, it appeared that results of yeast identification by MALDI-TOF MS were comparable regardless of whether the cultures were incubated for 48 or 72 h prior to protein extraction.

The major contribution of the present study is that it demonstrated the reliability of yeast protein extraction from a single colony instead of five, as recommended so far, making MS identification more suitable for the routine identification of yeasts in medical laboratories. It also demonstrated that either 48 or 72 h of preincubation on CHROMagar at 30°C is suitable for the correct identification of yeasts.

Other authors correctly identified 92.5 and 96.3% of their isolates by using a yeast protein extraction protocol based on the sampling of several colonies (3, 6). Similarly in our study, extraction of yeast proteins from a single colony allowed the correct identification of 94% of the isolates tested, allowing the validation of (i) yeast protein extraction from a single colony and (ii) the modification of the extraction protocol described here.

All of the isolates tested were obtained from clinical samples where mixtures of several yeast species often make the correct identification of yeasts difficult when sampling several colonies. Using only a single colony for the extraction step is a crucial point in medical laboratory practice, avoiding contamination with neighboring species responsible for misidentification of the yeast of interest. Moreover, clinical samples may harbor a very poor inoculum of yeasts, rendering the sampling of five single colonies difficult. In both cases, using a single colony overcomes these drawbacks.

Furthermore, after 72 h of preincubation on CHROMagar at 30°C, 97.3% of the clinical isolates tested were correctly identified. These results were comparable to those obtained after 48 h of preincubation. Thus, a 72-h preincubation period can be used instead of 48 h without affecting the accuracy of the identification. A preincubation period of 24 h was also tested and gave good identification scores (data not shown). However, this is not suitable in routine practice since the typical color of the colonies growing in chromogenic medium—necessary for detection of mixtures of yeast species in clinical samples—is rarely obtained after a 24-h preincubation time. Finally, being able to use both 48 and 72 h of preincubation before extracting yeast proteins provides flexibility without affecting the efficacy of MALDI-TOF MS identification, which is a considerable advantage in routine practice, especially when everyday MS identification is not possible (e.g., on Sundays or national holidays).

MALDI-TOF MS has already been shown to be a rapid and reliable tool for the accurate identification of *Candida* isolates that requires a minimum amount of time for interpretation of the results. In the present study, we proved the flexibility of the procedure, which makes it more suitable for routine analyses in a medical laboratory.

REFERENCES

1. **Bader O, et al.** 2011. Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin. Microbiol. Infect. **17**:1359–1365.
2. **Chen YC, et al.** 2000. Identification of medically important yeasts using PCR-based detection of DNA sequence polymorphisms in the internal transcribed spacer 2 region of the rRNA genes. J. Clin. Microbiol. **38**: 2302–2310.
3. **Dhiman N, Hall L, Wohlfiel SL, Buckwalter SP, Wengenack NL.** 2011. Performance and cost analysis of matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine identification of yeast. J. Clin. Microbiol. **49**:1614–1616.
4. **Ferroni A, et al.** 2010. Real-time identification of bacteria and *Candida* species in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **48**:1542–1548.
5. **Marinach-Patrice C, et al.** 2010. Rapid species diagnosis for invasive candidiasis using mass spectrometry. PLoS One **5**(1):e8862.
6. **Marklein G, et al.** 2009. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J. Clin. Microbiol. **47**:2912–2917.
7. **Miceli MH, Diaz JA, Lee SA.** 2011. Emerging opportunistic yeast infections. Lancet Infect. Dis. **11**:142–151.
8. **Odds FC, Bernaerts R.** 1994. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol. **32**:1923–1929.
9. **Pinto A, et al.** 2011. Matrix-assisted laser desorption ionization-time of flight mass spectrometry identification of yeasts is contingent on robust reference spectra. PLoS One **6**(10):e25712.
10. **Seyfarth F, et al.** 2012. Identification of yeast isolated from dermatological patients by MALDI-TOF mass spectrometry. Mycoses **55**(3):276–280.
11. **Verweij PE, Breuker IM, Rijs AJ, Meis JF.** 1999. Comparative study of seven commercial yeast identification systems. J. Clin. Pathol. **52**:271–273.