Protein Extraction of Non-*Candida* Yeast using an advanced acoustic technology for MALDI-TOF Identification

R. Lee[2], H. Miller[1], R. Green[2], N.P. Kwiatkowski[2], B. Ellis[2] and S.X. Zhang[1,2]

[1] Division of Medical Microbiology, Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland;

[2] Microbiology Laboratory, The Johns Hopkins Hospital, Baltimore, Maryland

Richard Lee
RLee72@jhmi.edu

Abstract

Background: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) has changed the way we identify yeast in the clinical labs. While correct identification of most common *Candida* species by MALDI-TOF can be achieved by using a simple and rapid on-target-lysis method, other yeasts must go through a full protein extraction process in order to be well characterized by the MALDI-TOF MS instrument. Bruker Daltonics' multi-step full protein extraction procedure has proven to be time-consuming. In this study, we explored an alternative method of protein extraction using Adaptive Focused Acoustics™ technology. This will allow us to generate spectrum with less hands on time using a focused-ultrasonicator (Covaris® M220, Woburn, MA, USA).

Materials/Methods: Representatives of 7 different yeast isolates include *Cryptococcus neoformans*, *C. gattii*, *Trichosporon mycotoxinivorans*, *Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae*, *Malassezia pachydermatis*, and *Saprochaete capitata* (formerly *G. capitatum*). Each isolate was extracted using Bruker's full protein extraction in parallel with Covaris' ultrasonication method. Different settings of the Covaris' ultrasonicator included Peak Incident Power (PIP) (75 vs 40 units), Duty Factor (DF) (25% vs 50%), type of microtubes (fiber vs bead), and ultrasonication time (1, 2, 3 min) were tested to optimize the efficacy of the Covaris extraction. The most effective combination was determined by the MALDI-TOF MS identification scores.

Results: Using a combination setting of 40 PIP, 50% DF, fiber tube and 1-2 min ultrasonication produced the most optimal extraction. At this setting, 5 of the 7 isolates (*C. neoformans*, *C. gattii*, *Trichosporon*, *Rhodotorula*, *Saccharomyces)* identified correctly with a score ≥2.0, comparable to Bruker's full extraction method. The remaining two, *M. pachydermatis* and *Saprochaete capitata* (formerly *G. capitatum*), gave "no reliable identification" by both methods.

Conclusion: Hands on time for Bruker' full extraction protocol took multiple steps and an average of 20 minutes for a single isolate. In contrast, the Covaris ultrasonication extraction method took fewer steps and averaged 1-2 minutes to achieve comparable MALDI-TOF MS scores. The Covaris' ultrasonication method proved to be a simple, rapid and efficient extraction tool and can be used in conjunction with MALDI-TOF MS for yeast identification.

Introduction

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) technology has changed the future of micro-organism identification from 24 hours to a few minutes with a simple spot of micro-organism and matrix. But, some organisms need an extra step to break the cell wall with formic acid. When scores are still unacceptable, a formic acid/acetonitrile tube extraction is needed. Unfortunately, a few minutes for identification turns into a 20 minute extraction for one isolate. While most common Candida species identifications can be achieved by direct plate extraction. We turned our focus to non-Candida species isolates that potentially would need the formic acid/acetonitrile tube extraction and explored a novel method of extraction using the Covaris M220 Focused-ultrasonicator™. We attempted to maximize protein extraction with the ultrasonicator by evaluating 4 different variables with 8 different non-*Candida species* . Protein extraction of each combination of variables were run on the MALDI-TOF in duplicate. The identifications scores were then used as a measurement of protein extraction.

Methods

A total of 7 different yeast non-*Candida species and 1 Candida albicans* (control) were used to evaluate 12 combinations of ultrasonication settings. Each isolate was run to compare the following variations:

- Beads vs Fiber
- 75 Peak Incident Power (PIP, max) and 25% Duty Factor or Cycle (D.F.) vs 40 PIP and 50% D.F. (max).
- Time (1, 2, and 3 minutes)

Methods (cont'd)

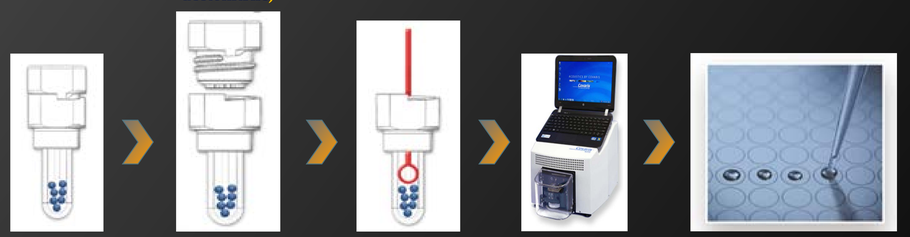
STEP 1: Centrifuge microTUBE prefilled with beads

STEP 2: Add 55 µL Extraction solvent 50:50 (70% formic acid : acetonitrile)

STEP 3: Add organism and repeat STEP 2 for additional 55 µL of solvent

STEP 4: Adaptive Focused Acoustics (AFA)

STEP 5: MALDI-TOF Spot 1µL product on target. Let dry. Overlay with matrix.



3,000 RCF for 10 seconds to pellet beads

1µL inoculum

Results

Table 1. Covaris Extraction Method Combinations

Isolate		Method	MALDI ID and Score											
		Time:	1 min				2 min				3 min			
		Duplicate Spotting:	Spot 1		Spot 2		Spot 1		Spot 2		Spot 1		Spot 2	
Candida albicans (Control)	Beads	75 PIP, 25% D.F.	*C. albicans*	1.989	*C. albicans*	2.129	*C. albicans*	1.875	*C. albicans*	1.825	No reliable ID	1.593	*C. albicans*	1.754
		40 PIP, 50% D.F.	*C. albicans*	2.065	*C. albicans*	1.991	*C. albicans*	1.826	*C. albicans*	1.835	*C. albicans*	1.738	No reliable ID	1.387
	Fiber	75 PIP, 25% D.F.	*C. albicans*	2.034	*C. albicans*	2.050	*C. albicans*	1.725	*C. albicans*	1.968	No reliable ID	1.441	No reliable ID	1.634
		40 PIP, 50% D.F.	*C. albicans*	1.907	*C. albicans*	2.123	*C. albicans*	1.985	*C. albicans*	1.814	No reliable ID	1.524	No reliable ID	1.692
Cryptococcus neoformans	Beads	75 PIP, 25% D.F.	*C. neoformans*	2.293	*C. neoformans*	2.348	*C. neoformans*	2.201	*C. neoformans*	2.230	*C. neoformans*	2.222	*C. neoformans*	2.178
		40 PIP, 50% D.F.	*C. neoformans*	2.189	*C. neoformans*	2.260	*C. neoformans*	2.347	*C. neoformans*	2.327	*C. neoformans*	2.277	*C. neoformans*	2.232
	Fiber	75 PIP, 25% D.F.	*C. neoformans*	2.302	*C. neoformans*	2.263	*C. neoformans*	2.268	*C. neoformans*	2.308	*C. neoformans*	2.361	*C. neoformans*	2.317
		40 PIP, 50% D.F.	*C. neoformans*	2.201	*C. neoformans*	2.217	*C. neoformans*	1.918	*C. neoformans*	1.992	*C. neoformans*	1.748	*C. neoformans*	2.206
Trichosporon mycotoxinivorans	Beads	75 PIP, 25% D.F.	*T. mycotoxinivorans*	1.859	*T. mycotoxinivorans*	1.884	*T. mycotoxinivorans*	1.772	*T. mycotoxinivorans*	1.807	No reliable ID	1.66	No reliable ID	1.407
		40 PIP, 50% D.F.	*T. mycotoxinivorans*	1.939	*T. mycotoxinivorans*	1.963	*T. mycotoxinivorans*	1.863	*T. mycotoxinivorans*	1.936	No reliable ID	1.667	No reliable ID	1.607
	Fiber	75 PIP, 25% D.F.	*T. mycotoxinivorans*	1.955	*T. mycotoxinivorans*	2.103	*T. mycotoxinivorans*	1.849	*T. mycotoxinivorans*	1.764	*T. mycotoxinivorans*	1.729	*T. mycotoxinivorans*	1.892
		40 PIP, 50% D.F.	*T. mycotoxinivorans*	2.156	*T. mycotoxinivorans*	2.093	*T. mycotoxinivorans*	2.278	*T. mycotoxinivorans*	2.139	*T. mycotoxinivorans*	2.027	*T. mycotoxinivorans*	1.853
Rhodotorula mucilaginosa	Beads	75 PIP, 25% D.F.	*R. mucilaginosa*	2.321	*R. mucilaginosa*	2.265	*R. mucilaginosa*	2.197	No peaks	<0	*R. mucilaginosa*	1.774	*R. mucilaginosa*	2.036
		40 PIP, 50% D.F.	*R. mucilaginosa*	2.356	No peaks	<0	*R. mucilaginosa*	2.223	*R. mucilaginosa*	2.272	*R. mucilaginosa*	1.89	*R. mucilaginosa*	2.138
	Fiber	75 PIP, 25% D.F.	*R. mucilaginosa*	2.387	No peaks	<0	*R. mucilaginosa*	2.398	*R. mucilaginosa*	2.460	*R. mucilaginosa*	2.387	*R. mucilaginosa*	2.342
		40 PIP, 50% D.F.	*R. mucilaginosa*	2.447	*R. mucilaginosa*	2.544	*R. mucilaginosa*	2.428	*R. mucilaginosa*	2.488	No peaks	<0	*R. mucilaginosa*	2.357
Cryptococcus gattii	Beads	75 PIP, 25% D.F.	*C. gattii*	2.092	*C. gattii*	2.173	*C. gattii*	2.119	*C. gattii*	2.058	*C. gattii*	2.050	*C. gattii*	2.086
		40 PIP, 50% D.F.	*C. gattii*	2.214	*C. gattii*	2.118	*C. gattii*	2.115	*C. gattii*	2.016	*C. gattii*	2.128	*C. gattii*	1.973
	Fiber	75 PIP, 25% D.F.	*C. gattii*	2.239	*C. gattii*	2.252	*C. gattii*	2.184	*C. gattii*	2.273	*C. gattii*	2.227	*C. gattii*	2.330
		40 PIP, 50% D.F.	*C. gattii*	2.023	*C. gattii*	2.155	*C. gattii*	2.178	*C. gattii*	2.204	*C. gattii*	2.122	*C. gattii*	2.362
Saccharomyces cerevesiae	Beads	75 PIP, 25% D.F.	*S. cerevesiae*	1.852	*S. cerevesiae*	2.095	*S. cerevesiae*	2.069	*S. cerevesiae*	2.013	*S. cerevesiae*	1.771	*S. cerevesiae*	1.852
		40 PIP, 50% D.F.	*S. cerevesiae*	1.837	*S. cerevesiae*	1.85	*S. cerevesiae*	2.024	*S. cerevesiae*	2.123	*S. cerevesiae*	2.058	*S. cerevesiae*	1.935
	Fiber	75 PIP, 25% D.F.	*S. cerevesiae*	2.021	*S. cerevesiae*	2.035	*S. cerevesiae*	2.096	*S. cerevesiae*	2.170	*S. cerevesiae*	2.057	*S. cerevesiae*	2.101
		40 PIP, 50% D.F.	*S. cerevesiae*	2.034	*S. cerevesiae*	1.938	*S. cerevesiae*	2.052	*S. cerevesiae*	2.009	*S. cerevesiae*	1.995	*S. cerevesiae*	1.906

Score color key: Green = ≥2.0, Yellow = 1.700 to 1.999, Red = <1.700, No Reliable Identification, or No Peaks

Table 2. Bruker Extraction Method

Isolate	Method	MALDI ID and Score			
	Duplicate Spotting:	Spot 1		Spot 2	
Candida albicans	Plate Extraction:	*C. albicans*	1.926	*C. albicans*	1.881
	Tube Extraction:	*C. albicans*	2.113	*C. albicans*	2.191
Cryptococcus neoformans	Plate Extraction:	*C. neoformans*	2.002	*C. neoformans*	2.151
	Tube Extraction:	*C. neoformans*	2.256	*C. neoformans*	2.248
Trichosporon mycotoxinivorans	Plate Extraction:	*No Peaks*	<0	*No Peaks*	<0
	Tube Extraction:	*T. mycotoxinivorans*	2.202	*T. mycotoxinivorans*	2.121
Rhodotorula mucilaginosa	Plate Extraction:	*R. mucilaginosa*	2.134	*R. mucilaginosa*	2.328
	Tube Extraction:	*R. mucilaginosa*	2.635	*R. mucilaginosa*	2.606
Cryptococcus gattii	Plate Extraction:	*C. gattii*	2.199	*C. gattii*	2.058
	Tube Extraction:	*C. gattii*	2.061	*C. gattii*	1.901
Saccharomyces cerevesiae	Plate Extraction:	*S. cerevesiae*	2.065	*S. cerevesiae*	1.932
	Tube Extraction:	*S. cerevesiae*	2.161	*S. cerevesiae*	2.265

Table 3. Protocol Variation Analysis

MALDI SCORE	Time (48)			1 minute Adaptive Focused Acoustics (24)		AFA setting - Fiber (12)	
	1 min	2 min	3 min	Beads	Fibers	75 PIP, 25% D.F.	40 PIP, 50% D.F.
≥2.0	70.8% (34/48)	64.6% (31/48)	47.9% (23/48)	58.3% (14/24)	83.3% (20/24)	83.3% (10/12)	83.3% (10/12)
1.700 to 1.999	25.0% (12/48)	33.3% (16/48)	29.2% (14/48)	37.5% (9/24)	12.5% (3/24)	8.3% (1/12)	16.7% (2/12)
<1.700	------	------	20.8% (10/48)	------	------	-----	-----
NP	4.1% (2/48)	2.1% (1/48)	2.1% (1/48)	4.2% (1/24)	4.2% (1/24)	8.3% (1/12)	-----

Conclusion

- Of the 7 non-*Candida species* isolates, *M. pachydermatis* and *Saprochaete capitata* (formerly *G. capitatum*) gave "no reliable scores" for both the Covaris and Bruker method and were omitted for comparison.
- The average time per isolate for Bruker's tube extraction method had a hands on time of ~20 minutes (including pellet drying). The Covaris method averaged ~2 minutes per isolate with spotting directly from the microTUBE after AFA disruption. Both methods gave comparable identification scores.
- The Covaris and Bruker plate extraction method are both similar in extraction time and identification scores. Conversely, the plate extraction for *Trichosporon mycotoxinivorans* gave "No peaks".
- From Table 3, the ideal settings using the Covaris ultrasonicator would be the following:
  - Material: Fiber
  - Time: 1 minute
  - AFA Setting: 40 PIP, 50% D.F.

Acknowledgement

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