EVALUATION OF THE PERFORMANCE OF MALDI-TOF-MS BIOTYPING TO IDENTIFY GIGASPORACEAE

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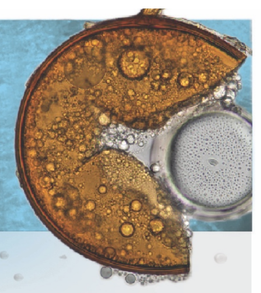


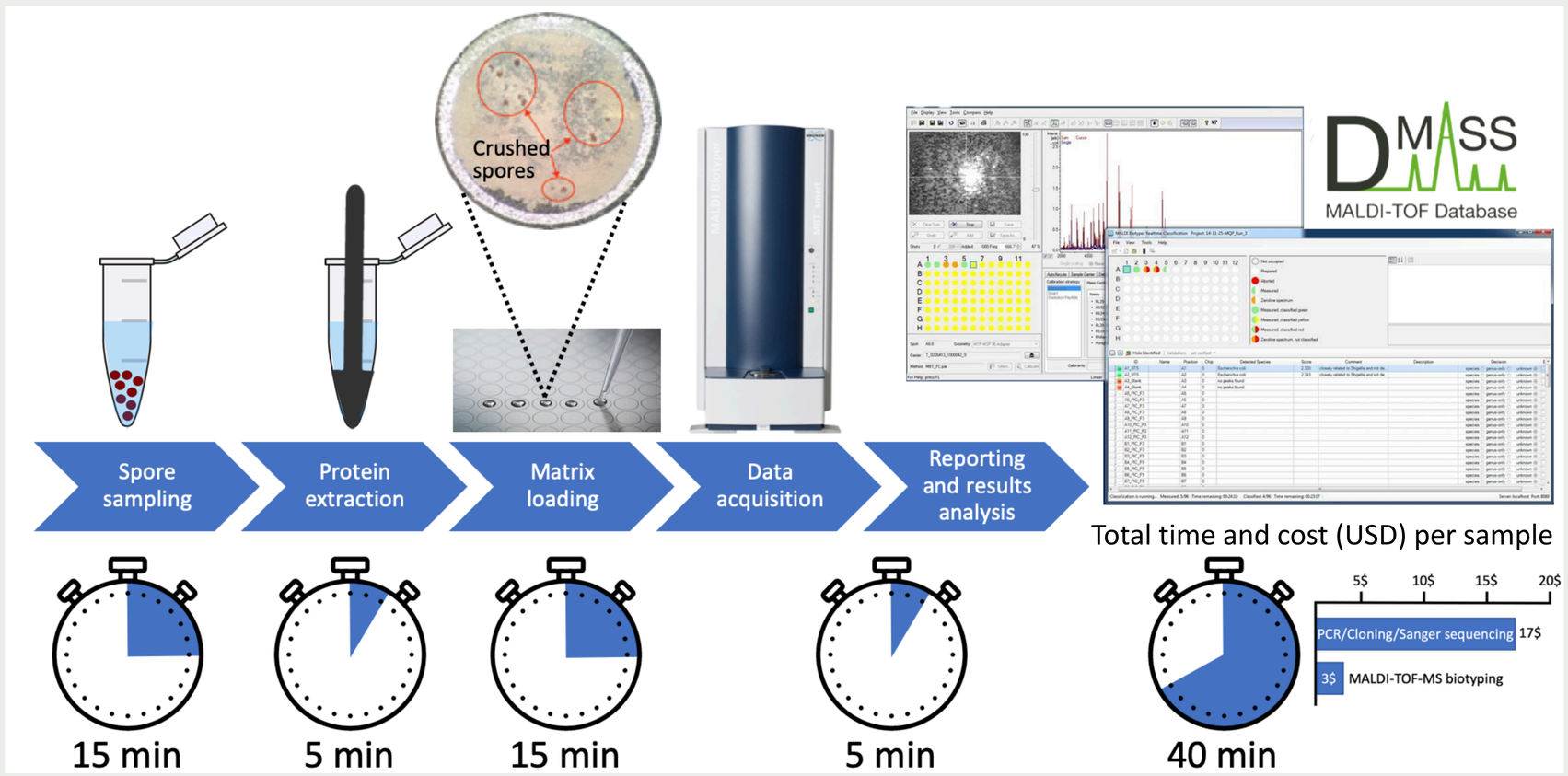
❑ BACKGROUND AND OBJECTIVES

The identification of arbuscular mycorrhizal fungi (AMF) is challenging due to limited morphological characters often obscured by homoplasy. So far, the amplification, cloning and sequencing of the 18S-ITS-28S region of the nuclear ribosome is the most reliable approach to identify species within AMF. Nevertheless, the entire molecular process is time-consuming and can be relatively expensive for routine identification.

Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) is a quick and reliable method for identification of microorganisms. As part of a research project between the International Bank of Glomeromycota (IBG), the Canadian Collection of Arbuscular Mycorrhizal Fungi (CCAMF) and University of New Caledonia (UNC), we investigate here the potential of MALDI-TOF MS biotyping to identify 20 isolates of Gigasporaceae (AMF). This family was targeted because some species within Gigasporaceae are genetically quite close and notoriously difficult to separate using molecular approach.

❑ METHODS

Figure 1. Steps of MALDI-TOF-MS biotyping. **Spore sampling:** 1 to 3 spores per strain are collected and cleaned up by sonication. Spores are transferred in 1.5 ml tubes containing 20 µL of ultrapure water. **Protein extraction:** spores are centrifuged, the supernatant is replaced with 10 µL of 70% formic acid and spores are crushed with a micropestle. **Matrix loading:** 1.5 µL of the supernatant is loaded on a polished steel MSP 96 target (Bruker) until the supernatant is used up.



❑ RESULTS

Genus	Species/isolates	No spores	MP score value	Organism (best match/second best match>1.3)
Cetraspora	*C. gilmorei 4436*	3	1.37	*C. gilmorei 4436*
		3	1.88	*C. gilmorei 4436*
	C. nodosa BEG4	3	1.3	*C. nodosa BEG4*
		3	0	*no peaks found*
	C. pellucida WV935	2	1.6	*C. pellucida WV935*
		2	1.72	*C. pellucida WV935*
	C. pellucida BEG238	2	1.88	*C. pellucida BEG238*
		2	1.87	*C. pellucida BEG238*
Dentiscutata	*D. heterogama CL157*	2	1.31	*D. heterogama CL157*
		2	1.76/1.11	*D. heterogama CL157/D. heterogama BEG35*
	D. heterogama BEG35	2	1.64	*D. heterogama BEG35*
		2	1.77	*D. heterogama BEG35*
Gigaspora	*G. albida CU131*	1	1.9	*G. albida CU131*
		1	0	*no peaks found*
	G. candida BEG17	1	2.3	*G. candida BEG17*
		1	2.42	*G. candida BEG17*
	G. gigantea BEG251	1	2.17	*G. gigantea BEG251*
		1	1.95	*G. gigantea BEG251*
	G. margarita 4292	1	1.29	*G. margarita 4292*
		1	1.45	*G. margarita 4292*
	G. margarita BEG34	1	2.25	*G. margarita BEG34*
		1	2.14	*G. margarita BEG34*
	G. rosea BEG9ca	1	1.33/1.20	*G. rosea BEG9ca/G. rosea BEG9*
		1	0	*no peaks found*
	G. rosea BEG9	1	2.37/1.6	*G. rosea BEG9/G. rosea BEG9ca*
		1	2.29/1.37	*G. rosea BEG9/G. rosea BEG9ca*
Racocetra	*R. castanea BEG1*	2	2.06	*R. castanea BEG1*
		2	2.01	*R. castanea BEG1*
	R. fulgida NC303A	3	0	*no peaks found*
		3	0	*no peaks found*
	R. verrucosa HA150A	1	1.32	*R. verrucosa HA150A*
		1	0	*no peaks found*
Scutellospora	*S. calospora IL209*	2	1.61	*S. calospora IL209*
		2	1.44	*S. calospora IL209*
	S. calospora BEG245	2	1.45	*S. calospora BEG245*
		2	1.99	*S. calospora BEG245*
	S. calospora BEG32	2	2.27	*S. calospora BEG32*
		2	2.31	*S. calospora BEG32*
	S. ovalis	2	1.92	*S. ovalis*
		2	2.13	*S. ovalis*

Table 1. Spore identification of 20 isolates of Gigasporaceae by MALDI-TOF-MS biotyping: Bruker real-time analysis of log Main Pattern (MP) scores.

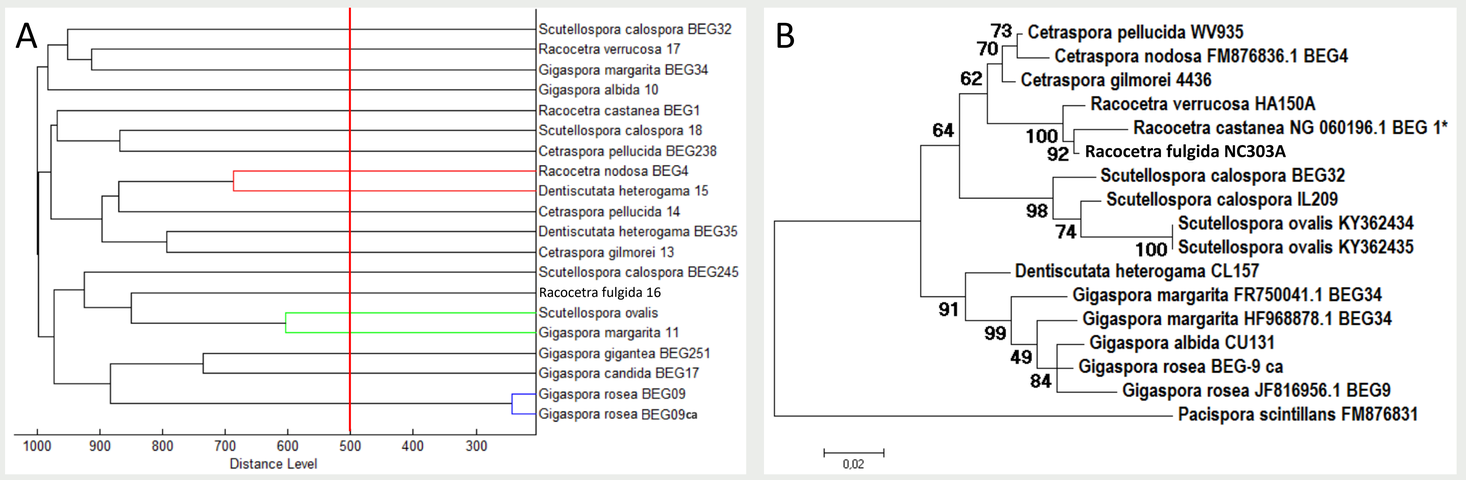


Figure 2. A) Cluster analysis of MALDI-TOF MS spectra, distance levels > 500 (represented by the red line) have been considered as reliably classified to the same species. B) Maximum likelihood (ML) phylogenetic tree based on 18S-ITS-28S rDNA sequences.

20 cultures (Table 1) were processed according to Fig 1. in a single working day and 19/20 could be reliably identified. Each isolate showed a unique proteome profile. MALDI-TOF-MS biotyping was able to separate closely related isolates (under species level) and to assign a unique fingerprint to *G. albida* CU131 which 18S-ITS-28S sequence shared more than 98% of similarity with *G. rosea* BEG-9. However, phylogenetic relationships (Fig.2B) between species and genera were not reliably recovered using proteomic profiles (Fig. 2A).

❑ CONCLUSION

MALDI-TOF-MS biotyping is fast, accurate and inexpensive and it represents a promising avenue for “routine” quality control of culture collections and of commercial biofertilizers.

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