TECHNICAL NOTE

#28 Optimization of DHB Matrix Spray for MALDI Imaging of Metabolites in Root Nodule Tissue of the *Medicago truncatula – Sinorhizobium meliloti* Symbiosis

Application

2,5-dihydroxybenzoic acid (DHB) is a matrix suitable for imaging metabolites. DHB can easily be dissolved in 50:50 Water:Methanol (0.1% Trifluoroacetic Acid) solution. When imaging metabolites DHB can be sprayed directly onto tissue sections with no pre-treatment, but may show interfering peaks in the low mass region of the mass spectra. The data presented here were obtained as part of a MALDI MS imaging experiment whose purpose was to detect metabolites present in the root nodules of the *Medicago truncatula – Sinorhizobium meliloti* symbiosis during nitrogen fixation.

Intended Use Of This Technical Note

The information provided in this document was not peer reviewed and is intended to illustrate possible uses of the TM-Sprayer. HTX Technologies, the manufacturers referenced in this note and the users that have accepted to share their data do not make any guarantees as to the performance of the illustrated workflow.

Imaging Workflow

Root nodules were dissected out of the plant, embedded in gelatin (100 mg/ml in deionized water), and frozen on dry ice. Cryosections (14 microns) of snap frozen root nodule tissue was mounted on ITO-coated glass slides.

Tissue sections were then sprayed with DHB matrix (40mg/ml, Methanol 50%, TFA 0.1%) using the HTX TM-Sprayer and the following conditions:

Flow Rate	50 µl/min
Spray Nozzle Velocity	1250 mm/min
Spray Nozzle Temperature	80°C
Track Spacing	3 mm
Number of Passes	24, criss-cross and offset
Nitrogen Pressure	10 psi

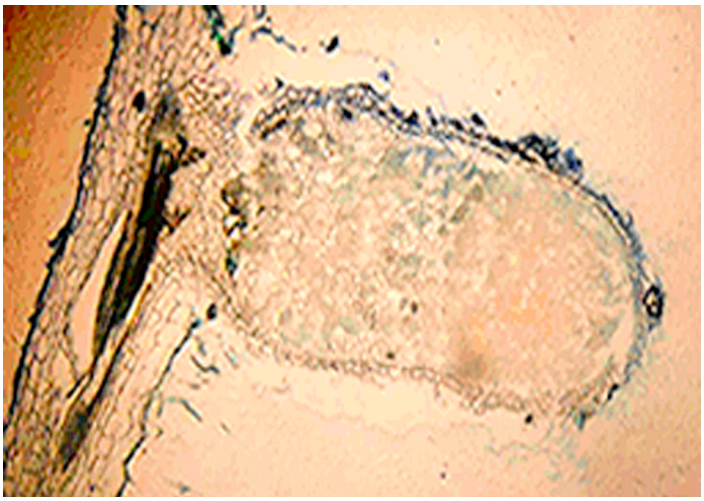


Figure 1. Methylene blue stained *Medicago truncatula* root nodule (Image courtesy of Dr. Jean-Michel Ané lab in the Department of Agronomy at UW-Madison.)

Spectra were collected across the entire tissue area using the ultrafleXtreme MALDI-TOF/TOF (Bruker Daltonics, Billerica, MA, USA) analyzer equipped with a 2 kHz, FlatTop smartbeam-II™ Nd:YAG laser in reflectron positive mode over a mass range of m/z 80 to 1000. A total of 500 laser shots were accumulated and averaged from each laser spot, using the "minimum" laser spot diameter setting and a raster width of 50µm. Calibration was performed externally using DHB cluster peaks in the mass range of m/z 100 to 750.

Experimental Summary

Tissue type	Root nodule
Preservation	Cryogenic storage
Tissue cut	14 µm thickness
MALDI Plate	Glass slide adapter
Matrix deposition	DHB 40mg/ml, 0.1% TFA in 50:50 MeOH/H_20
MALDI Laser	FlatTop smartbeam-II™ Nd:YAG laser
Acquisition mode	Reflectron positive

Instrumentation and Supplies

Microtome	Thermo Microm HM525
MALDI plate	ITO coated slides
Matrix	Acros Organics
Matrix Sprayer	HTX TM-Sprayer™
MALDI MS	BRUKER ultrafleXtreme™
Imaging software	BRUKER flexImaging

Acknowledgements

The tissue images and MS data presented in this note were provided by Erin Gemperline (Department of Chemistry) and Dr. Lingjun Li (Department of Chemistry and School of Pharmacy), University of Wisconsin-Madison, Madison, WI, USA

Results and MALDI MS Images

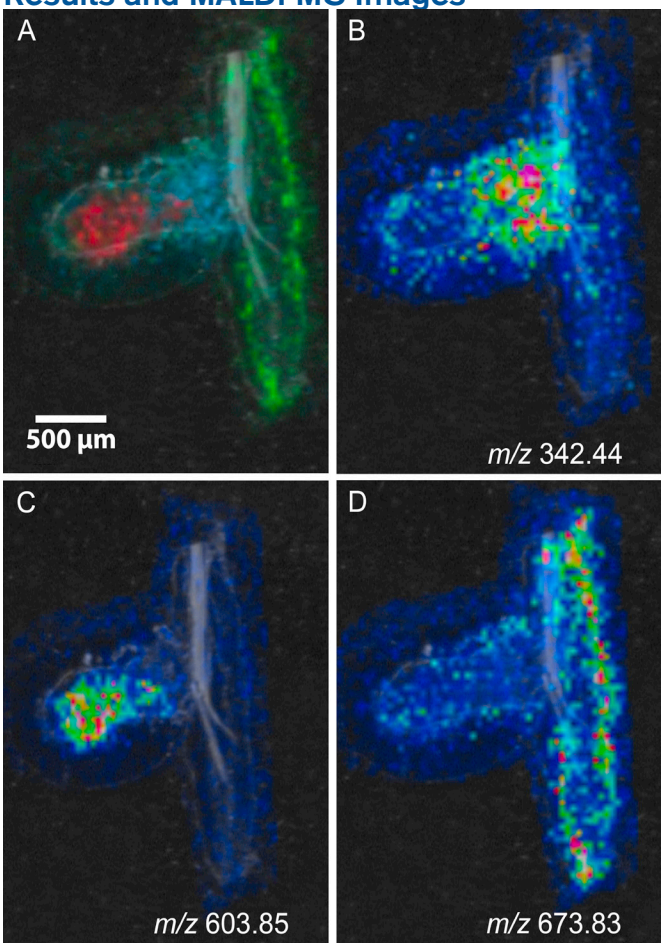


Figure 2. A) Overlaid images of m/z 342.44, 603.85, and 673.83 which show spatial differentiation in different parts of the root nodule. B) Distribution of m/z 342.44 localized to the nitrogen fixation zone region. C) Distribution of m/z 603.85 localized to the outer nodule region. D) Distribution of m/z 673.83 localized to the root region.

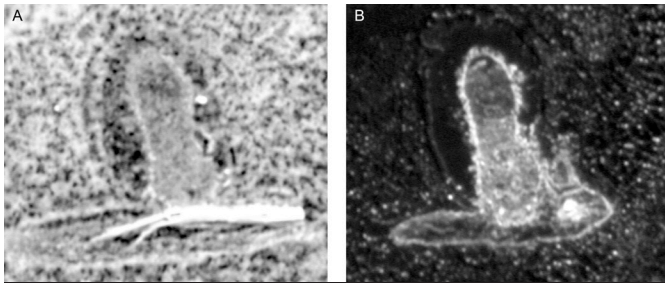


Figure 3. Reference Optical Image

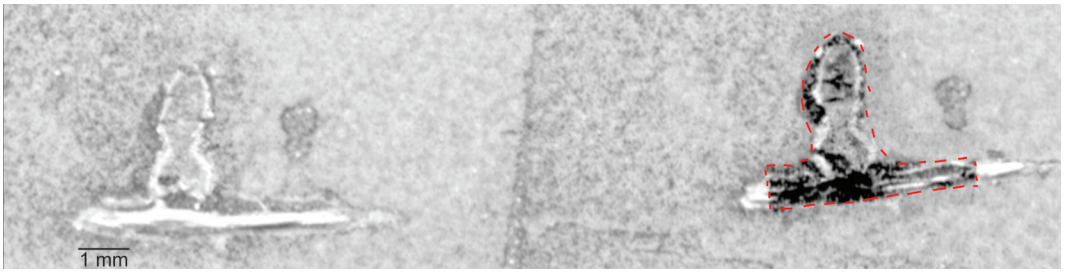


Figure 4. Optical image of serial tissue sections before (left) and after (right) MALDI- MSI. The area of tissue that was imaged is outlined in red on the right.

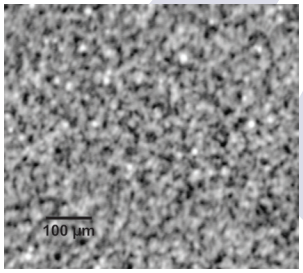


Figure 5. Zoomed in imaged of matrix crystals showing crystal size and coverage.

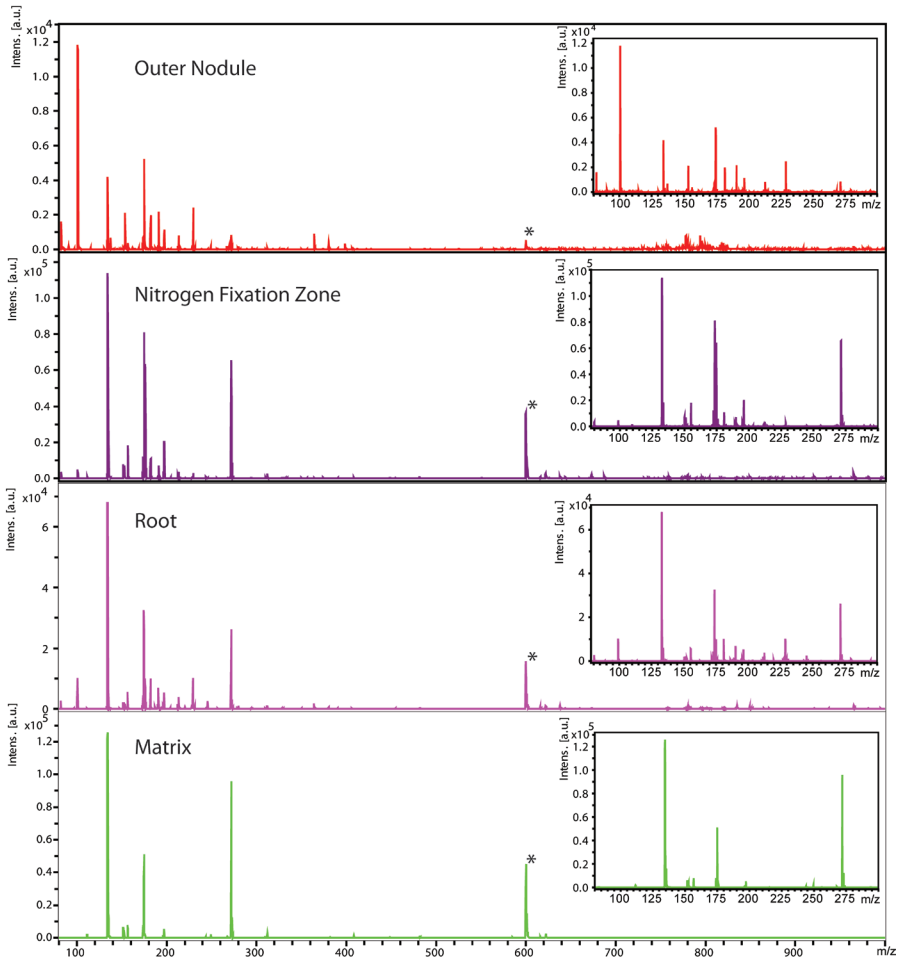
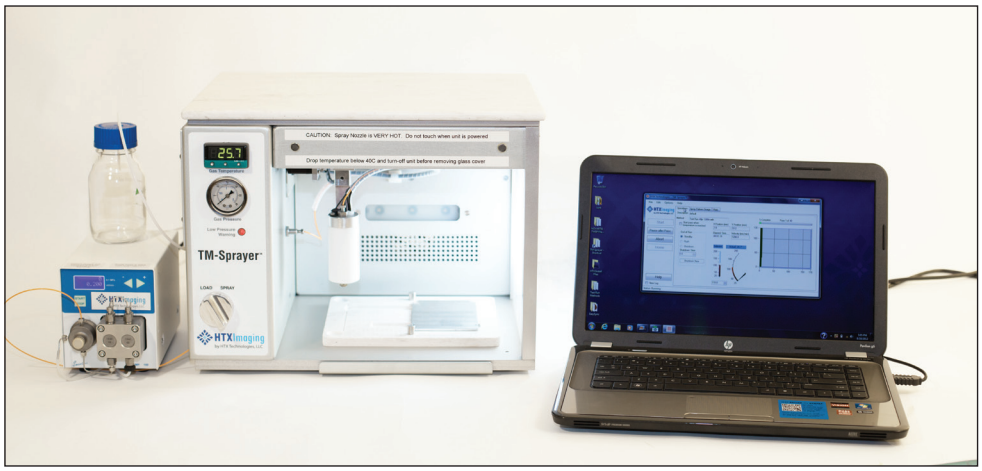


Figure 6. Mass spectra for the three distinct regions of the root nodule tissue: outer nodule, nitrogen fixation zone, and root. Metabolites with m/z 80-1000 were imaged. The inlay zooms in on the region m/z 80-300 which has the greatest abundance of different metabolites. The calculated [M+H]+ for FMRFamide is 599.3, which was added to the matrix as an internal calibrant.

Figure 7. *Medicago truncatula*- intact plant before dissection (Image courtesy of Dr. Jean-Michel Ané in the Department of Agronomy at UW-Madison.)

TM-Sprayer™ Tissue MALDI Sample Preparation System

The HTX TM-Sprayer™ System is an automated MALDI matrix deposition system offering high reproducibility and superior data quality for Mass Spectrometry Imaging



The HTX TM-Sprayer™ is an easy-to-use, versatile spraying system that provides an automated process for Sample Preparation in Mass Spectrometry Imaging.

The patented spray technology of the TM-Sprayer™ guarantees a very fine, uniform and consistent matrix coating crucial for high-resolution imaging and relative quantification of analytes.

The new HTX Technologies' spray nozzle, featured in the next generation TM-Sprayer, creates a fine solvent mist that can be deposited in a precise and adjustable pattern over all or part of any MALDI plate.

Spray characteristics (wet or dry) are easily adjustable via the intuitive operator interface. Users can create and save methods for reproducible operation.

Key Characteristics

- Patented technology providing very small matrix droplets (<10 microns)
- High flow rate and fast sample prep (10 to 20 minutes per plate)
- Highly consistent matrix deposition across entire sample area (+/- 3% by weight)
- Unique use of temperature and nitrogen flow to control evaporation rate and matrix crystal formation
- Validated protocols for most matrices (e.g.: SA, CHCA, DHB)
- Validated protocols for Trypsin digestion
- Continuous matrix coverage as needed for high-resolution imaging
- Rugged operation and easy clean-up

TM-Sprayer™ Specifications

Deposition: Spray deposition in linear or serpentine modes with variables offsets

Spray Nozzle Flow: 50 to 1000µl/min

Sheath Gas: Ambient to 150°C (+/- 2°C), software selected

Gas Supply: Sheath gas flow 5-15.5 liter/min

Spray Nozzle Position: Spray nozzle mounted on Cartesian stage

Electrical: 36V Power Supply

Dimensions/Weight: 17 x 15 x 13in (43 x 38 x 33cm), 38lbs (17Kg)

TM-Sprayer™ is available worldwide exclusively from HTX Technologies, LLC. To request further information contact:

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HTX Technologies offers innovative sample preparation systems for advanced analytical platforms. Our integrated workflow solutions include user training, instruments, software, consumables and method development services.

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