Structural characterization of membrane glycolipids from marine sponge-associated bacteria by mass spectrometry

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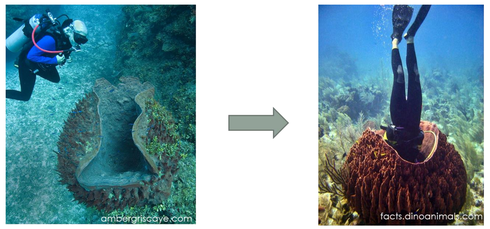
Introduction:

Many marine sponges, including the giant barrel sponge *Xestospongia muta*, harbor complex microbiomes and novel bacteria associated with these sponges have been isolated and classified by 16S rDNA sequencing [1]. Sponge-symbiont relationships are not yet well understood. It is clear, however, that symbionts are responsible for a portion of the production of bioactive metabolites previously isolated from sponges. Since membrane glycolipids, such as lipopolysaccharide (LPS) and lipoteichoic acid (LTA), play a vital role in bacterial recognition by host cells that possess a Toll-like receptor-mediated inflammation pathway [2], these molecules can be used to form a structure-activity relationship to describe the resultant host response to infection. Glycolipids produced by non-terrestrial bacteria were isolated and analyzed by mass spectrometry (MS) to improve the overall understanding of sponge-bacteria symbiosis.



Methods:

Bacterial species were isolated from *Xestospongia muta* or *Mycale laxissima* and cultured separately in either ISP2 or MA2216 medium. These cells were lysed and the membrane glycolipids were extracted and partially hydrolyzed according to the method described by El Hamidi, *et al* [3]. Extracts were lyophilized and re-dissolved in a 2:1:0.1 solution of chloroform, methanol, and water, respectively. Samples were analyzed first by negative ion mode MALDI-TOF on a Bruker Microflex using norharmane as matrix. Tandem mass spectra with ion mobility separation were acquired on a hybrid quadrupole ion mobility spectrometer-orthogonal acceleration time of flight (Q-IMS-oaTOF) mass spectrometer (Waters Synapt G2-S HDMS) operated in resolution mode with negative ionization. Collision energy was ramped from 5V to 100V to simulate an MS^n experiment. MALDI mass spectrometry imaging was performed at 75 µm spatial resolution on *Xestospongia muta* tissue sections using a Bruker Solarix XR 12T FT-ICR MS, operated in negative ion mode with norharmane as matrix – deposited on the target plate with a Sunchrom SunCollect automated sprayer.

Results:

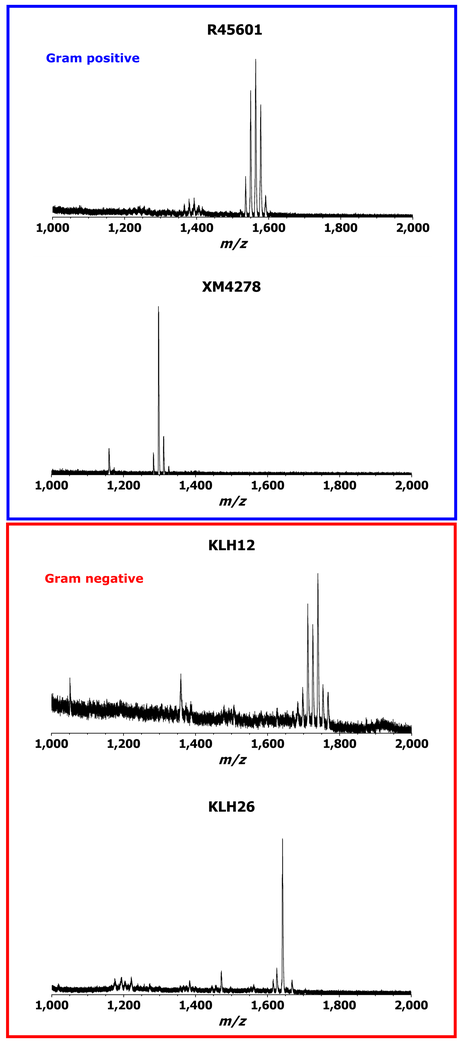


Figure 1. MALDI-TOF mass spectra of glycolipid extracts from four bacterial symbiont species. (blue box) Two spectra from Gram positives isolated from *X. muta*; putative identifications of major ions as glycolipid membrane anchors of LTA. (red box) Two spectra from Gram negatives isolated from *M. laxissima*; putative identifications of major ions as lipids A.

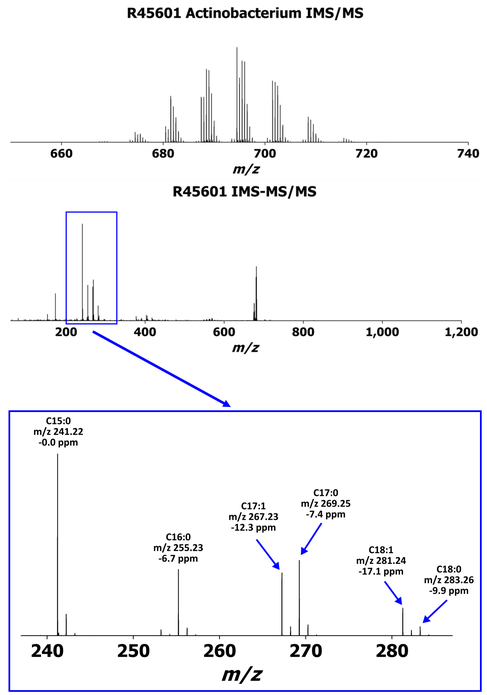


Figure 2. (top) ESI-IMS/MS of an extract from a representative Gram positive species (R45601, actinobacterium) with internal calibration; doubly charged ions with 14 Da spacing, corresponding to fatty acyl chain length discrepancies. (bottom) ESI-IMS-MS/MS of R45601 extract. (inset) Fatty acid product ions from precursor ion at *m/z* 674.5.

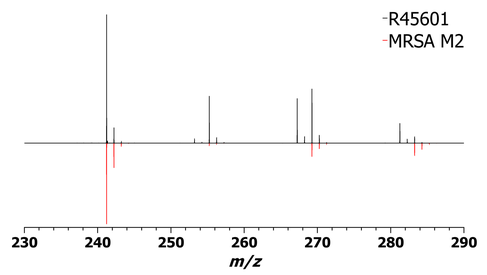


Figure 3. Comparison of fatty acid distribution between membrane glycolipid anchor of LTA from R45601 and M2 strain of methicillin-resistant *S. aureus*.

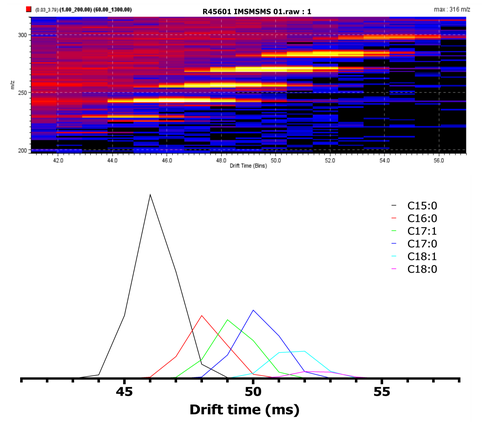


Figure 4. (top) Heat map from post-dissociation traveling wave ion mobility spectrometry experiment showing two dimensional separation of fatty acid product ions in both the mass-to-charge and drift time domains from LTA precursor. (bottom) Arbitrary intensity v. drift time plot extracted from above experiment.

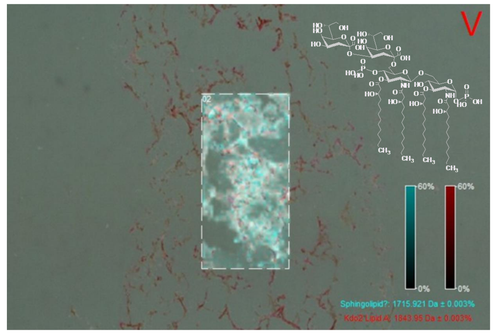


Figure 5. 75 µm spatial resolution MALDI mass spectrometry imaging of cryosectioned *X. muta* tissue. Kdo2-lipid A was putatively identified with accurate mass (Δ < 1 ppm). A glycosphingolipid (cerebroside) ion was used for contrast.

Discussion and Conclusions:

- Rapid screening of extracts for quality using MALDI-TOF MS allowed increased efficiency for structural sample analysis.
- Both Gram positive and Gram negative species' membrane glycolipids were extracted using the same sample preparation procedure. Glycolipid membrane anchor of LTA was isolated by acid hydrolysis, supported by previous work of Jang *et al*. [4].
- For both Gram positives and Gram negatives, fatty acids were observed in tandem mass spectra as product ions; an advantage to using a beam-type mass spectrometer over an ion trap for this type of analysis. Collision energy ramping was used to generate complete tandem mass spectra.
- Post-dissociation, fatty acids were separated using traveling wave ion mobility spectrometry. Clear trend observed.
- Imaging experiments resulted in putative identifications of bacterial symbiont glycolipids as well as host glycolipids.
- Efforts are ongoing to achieve complete structural assignment for glycolipids isolated from bacteria. This is a challenge due to extreme sample heterogeneity. Chromatographic separation method development is underway to decrease sample complexity.

Acknowledgment:

The authors would like to thank Dr. Alison Scott for assistance with MALDI mass spectrometry imaging of sponge tissues, and Lisa Leung for assistance with data analysis.

References:

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2. Chow, J.; Young, D.; Golenbock, D.; Christ, W.; Gusovsky, F. Toll-like Receptor-4 Mediates Lipopolysaccharide-Induced Signal Transduction. *The Journal of biological chemistry.* **1999**, *274*(16), 10689–10692.
3. Hamidi, E.; Tirsoaga, A.; Novikov, A.; Hussein, A.; Caroff, M. Microextraction of Bacterial Lipid A: Easy and Rapid Method for Mass Spectrometric Characterization. *Journal of lipid research.***2005**, *46* (8), 1773–1778.
4. Jang, K.; Baik, J.; Han, S.; Chung, D.; Kim, B. Multi-Spectrometric Analyses of Lipoteichoic Acids Isolated from Lactobacillus Plantarum. *Biochemical and biophysical research communications.* **2011**, *407* (4), 823–830.

Presentations from Goodlett Lab and Collaborators

TOF pm 02:30 Absorption Mode Processing of MALDI-FT-ICR Imaging Data Improves Mapping of Gram-Negative Bacterial Virulence Factors on-Tissue, Alison Scott
MP372 Bio-Molecule Characterization Using a Novel Ion Mobility Orbitrap Mass Spectrometer, Sung Hwan Yoon
MP440 Autopiquer – Introducing a New Approach to High Confidence Peak Detection, David Kilgour
MP712 Top-Down Mass Spectrometry Applications for Detection of N-Terminal Sequence Heterogeneity and PTMs for a Therapeutic Molecule, Young Ah Goo

TP040 Phosphoproteomic Analysis of Differential Protein Expression in BRaf-Mutated Melanoma Cells with Acquired Resistance to BRaf, MEK1/2, or ERK1/2 Inhibitors, Shivangi Awasthi
TP269 Validation of a GC/MS/MS Method for the Analysis of Chemical Warfare Agents (CWAs), Tom Rusek
TP506 Top-down Structural Elucidation of Gram-negative Bacterial Endotoxins by Tandem Mass Spectrometry, Mohsin Khan
TP507 Structural Characterization of Lipid Biomarkers from Staphylococcus aureus following Microextraction for Mass Spectrometric Phenotyping, Lisa Leung

ThP025 Surface Acoustic Wave Nebulization Sample Introduction for Vacuum-Assisted Plasma Ionization, Stephen Zambrzycki
ThP026 Surface Acoustic Wave Nebulization – Mass Spectrometry: A Tool for Rapid Analysis of Food Products, Gloria Yen
ThP027 Performance Characterization of Surface Acoustic Wave Nebulization for Lipid A Mass Spectrometric Analysis, Tao Liang
ThP028 Surface Acoustic Wave Nebulization – Mass Spectrometry on a TripleTOF Mass Spectrometer, David Goodlett
ThP209 A Rapid, Cell Culture-Based Method for Biomarker Discovery and Drug Screening by MALDI-MSI, Courtney Chandler

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