Characterization of Lipopolysaccharide Modifications in Select Antibiotic-resistant Gram-negative Bacteria using Surface Acoustic Wave Nebulization Mass Spectrometry

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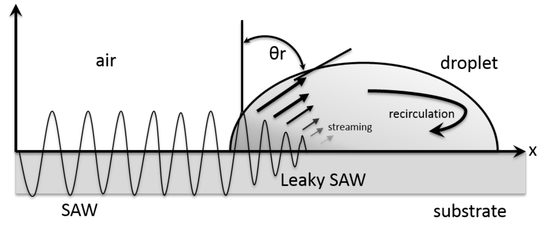
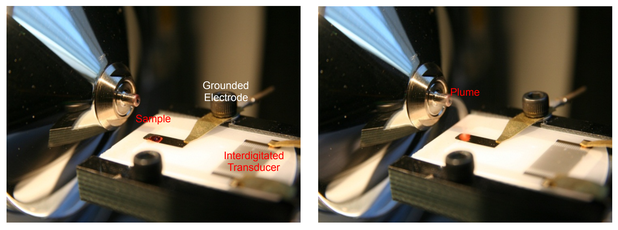
Introduction

Characteristic bacterial lipid mass spectrometric profiles based on antibiotic resistance have been demonstrated using MALDI and ESI MS analyses. There are limitations to these technologies: namely, the matrix interference signal and higher ionization energy of MALDI and the tendency of analytes such as lipids to aggregate and clog capillaries during ESI. Surface acoustic wave nebulization (SAWN) allows generation of ions without the use of matrices via an energetically softer ionization method than ESI, which preserves more precursor ions.

Surface Acoustic Wave Nebulization

SAWN technology utilizes surface acoustic waves (SAW) for nebulization directly from a solution deposited on the flat surface of a piezoelectric Lithium Niobate ($LiNbO_3$) wafer using radio frequency waves applied to the interdigitated transducer (IDT). MS analysis is performed on the Waters Synapt G2S in which the LC apparatus has been removed and the SAWN $LiNbO_3$ piezoelectric chip is mounted on a platform just beneath the capillary inlet. Nebulization generates a plume that is drawn into the mass spectrometer by the pressure gradient that exists with an atmospheric pressure ion interface.

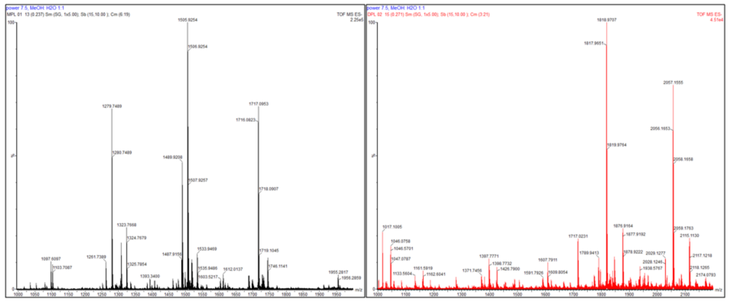




The SAWN-generated ions come directly from a planar surface simplifying the sample preparation process. Also, as an ambient ionization method, SAWN can limit uncontrolled fragmentation of the precursor ions. SAWN can be operated in continuous mode as in ESI or pulsed mode like MALDI depending on the sample introduction.

Analysis of lipid A by SAWN

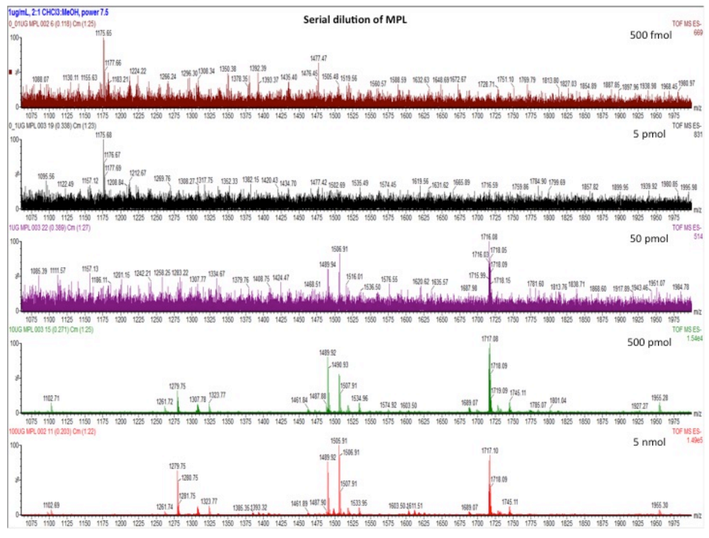
MPL from *Salmonella minnesota* DPL from *Escherichia coli* K-12



Synthetic mono- and diphosphoryl lipid A were obtained commercially and analyzed by SAWN. Approximately 50 nmol sample in 2:1 chloroform:methanol were deposited onto SAWN chip. Spectra were acquired in negative mode using Waters Synapt G2S™ HD MS.

Limit of Detection of Monophosphoryl Lipid A

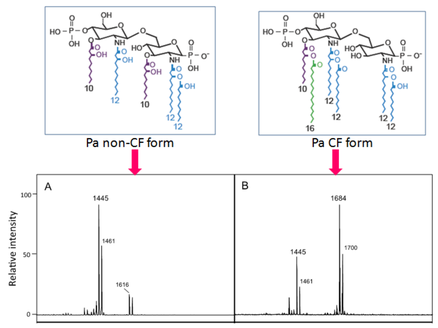
MS^1 of MPL LOD using SAWN



SAWN was acquired in negative mode using Waters Synapt G2S™ HD MS

Conclusions

- Mass spectrometry is able to produce unique lipid profiles for cationic antimicrobial peptide (CAMP)-resistant strains of *Pseudomonas aeruginosa* that permit us to distinguish these bacteria from susceptible strains



- SAWN can distinguish between closely related strains by lipid analysis of bacterial membranes
- SAWN can be utilized to characterize highly similar lipid A structures across different species
- SAWN is more advantageous than the most widely used current MS technologies in terms of ease of use
- Spectra generated using SAWN display fewer fragmentation peaks that are also generally of lower intensity than the more energetic ionization methods of ESI and MALDI

References

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3. Yoon et al., *Anal. Chem*. **84**, 6750-6757 (2012)

Acknowledgements

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