

Two-Dimensional Tandem Mass Spectrometry for Bacterial Profiling

L. Edwin Gonzalez, Lucas J. Szalwinski, Thomas C. Sams, Eric T. Dziekonski, R. Graham Cooks

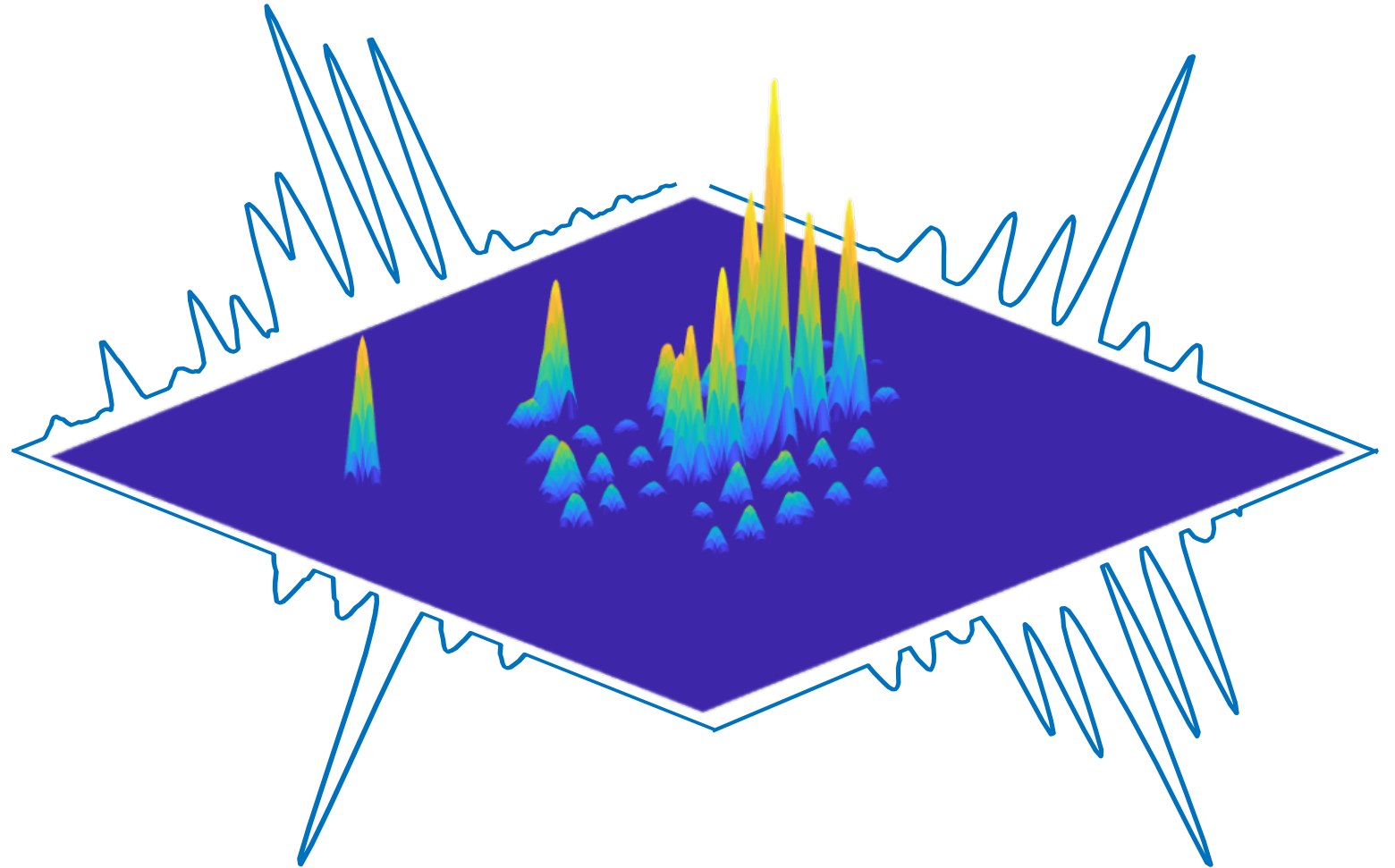
Department of Chemistry, Purdue University

Aston Laboratory for Mass Spectrometry Research




Introduction


- Motivation
- Sample Preparation
- Ion Trap Theory
- 2D MS/MS Analysis
- 2D Opportunities




Biological Threats




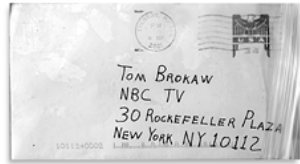
SPECIAL REWARD
Up to **\$2.5 million**




For information leading to the arrest and conviction of the individual(s) responsible for the mailing of letters containing anthrax to the New York Post, Tom Brokaw at NBC, Senator Tom Daschle and Senator Patrick Leahy:









**AS A RESULT OF EXPOSURE TO ANTHRAX,
FIVE (5) PEOPLE HAVE DIED.**

The person responsible for these deaths...

- Likely has a scientific background/work history which may include a specific familiarity with anthrax
- Has a level of comfort in and around the Trenton, NJ area due to present or prior association

Anyone having information, contact **America's Most Wanted at 1-800-CRIME TV** or the **FBI** via e-mail at **amerithrax@fbi.gov**

All information will be held in strict confidence. Reward payment will be made in accordance with the conditions of Postal Service Reward Poster 296, dated February 2000. Source of reward funds: U.S. Postal Service and FBI \$2,000,000; ADVO, Inc. \$500,000.

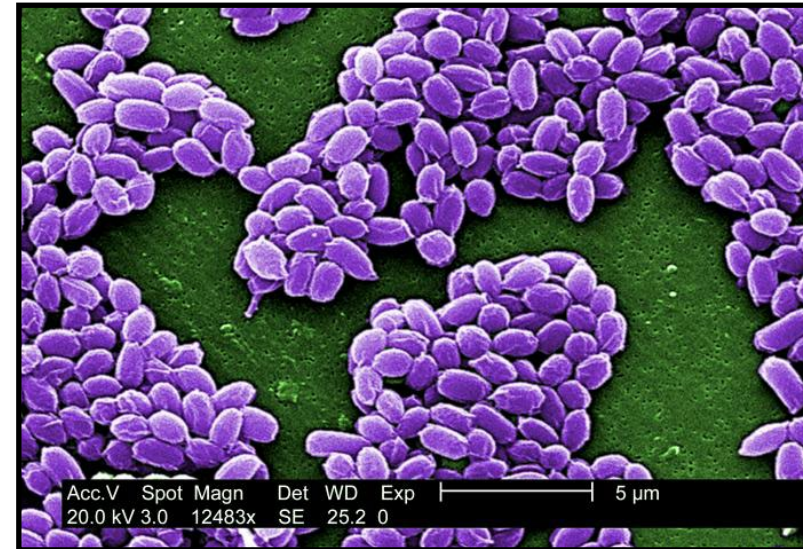


Table 1: Agents that can be used as biological warfare or bioterrorism-related incidents

	Agents	Disease	Route of infection	Possible release
Bacteria	<i>Ba. anthracis</i>	Anthrax	Aerosol	Spores
	<i>Y. pestis</i>	Plague	Aerosol	Vegetative cells
	<i>Br. melitensis</i>	Brucellosis	Aerosol	Vegetative cells
	<i>Br. abortus</i>			
	<i>Bu. mallei</i>	Glanders	Aerosol	Vegetative cells
Viruses	<i>Bu. pseudomallei</i>	Melioidosis	Aerosol	Vegetative cells
	Variola virus	Smallpox	Aerosol	Virus particles
	Ebola virus	Ebola hemorrhagic fever	Aerosol	Virus particles
	Marburg virus	Marburg hemorrhagic fever	Aerosol	Virus particles
Toxins	<i>C. botulinum</i>	Botulism	Ingestion food/water	Toxin
	<i>Staphylococcus aureus</i>	Staphylococcal enterotoxin type B (SEB)	Food/water	Toxin
	Ricin (plant)	Ricin toxin	Food/water	Toxin
	Trichothecene (fungus)	Trichothecene T2 toxin	Food/water	Toxin

1) <https://www.cdc.gov/anthrax/basics/types/index.html#:~:text=Inhalation%20anthrax%20starts%20primarily%20in,about%2055%25%20of%20patients%20survive.>

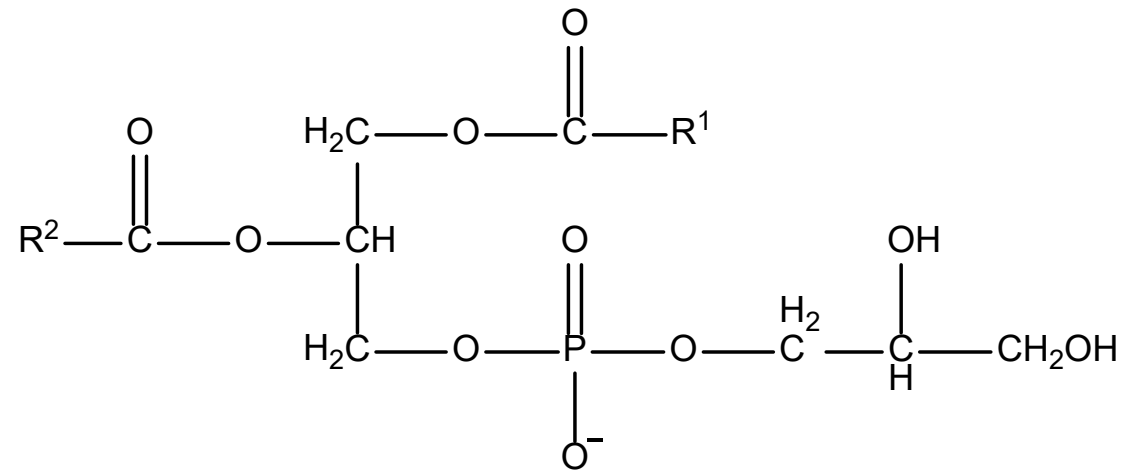
2) Thavaselvam D, Vijayaraghavan R. Biological warfare agents. J Pharm Bioallied Sci. 2010 Jul;2(3):179-88. doi: 10.4103/0975-7406.68499. PMID: 21829313; PMCID: PMC3148622.

3) https://en.wikipedia.org/wiki/2001_anthrax_attacks

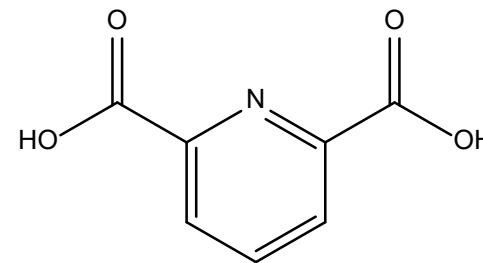
Bacterial Profiling

- *Bacillus* Genus Form Spores
- Each Species has a Unique Lipid Membrane
- Dipicolinic Acid is a Metabolic Product of Sporulation
- Lyse Spores with Microwave

Phosphatidylglycerol (PG)

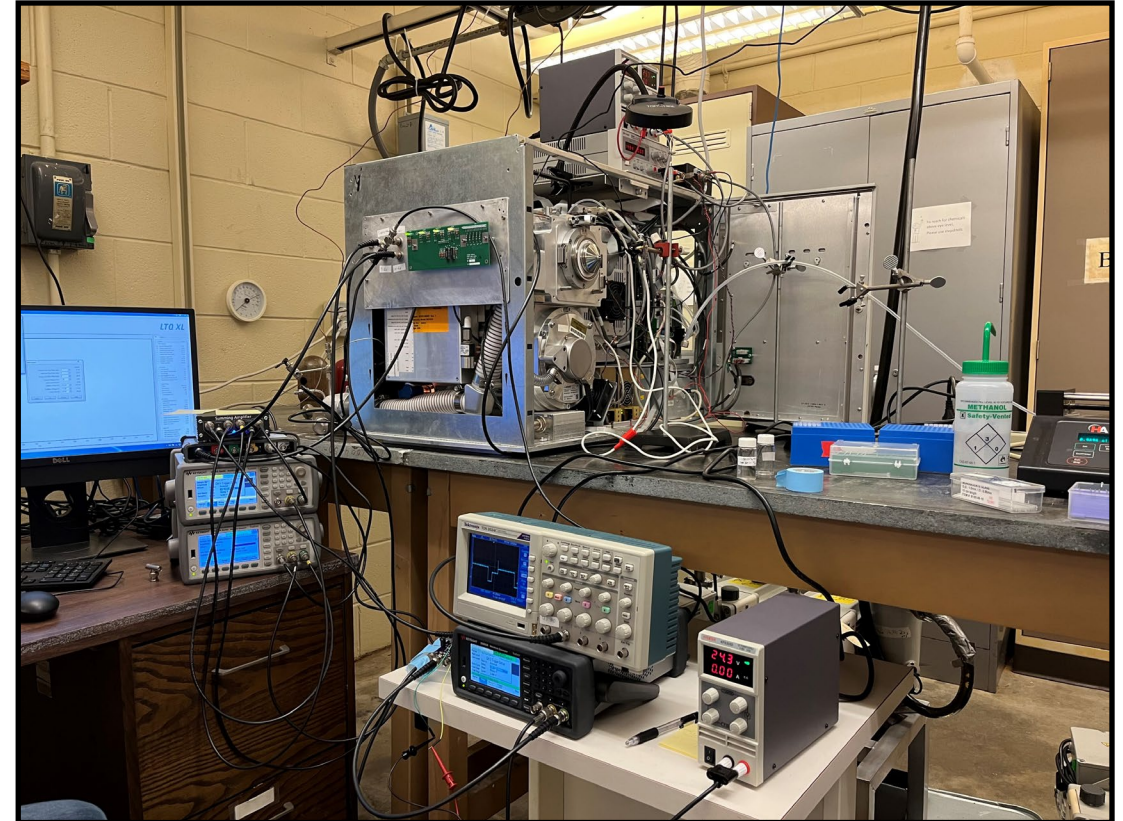


Dipicolinic acid

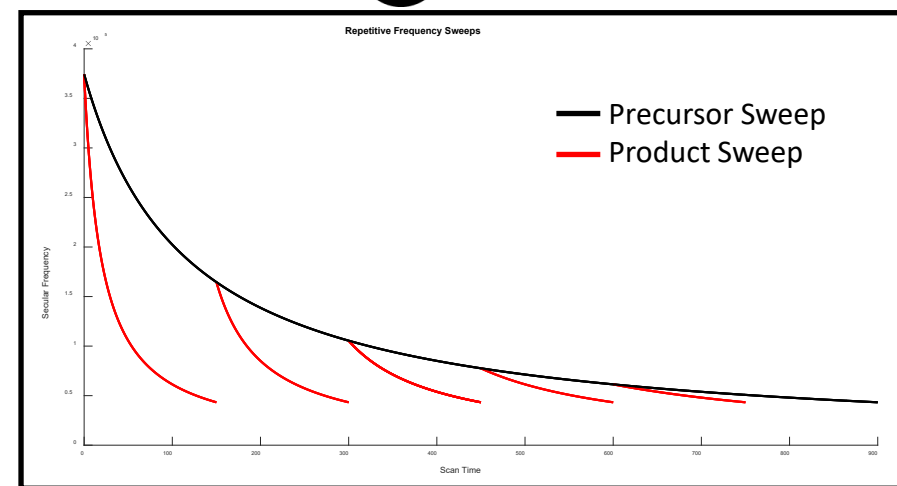
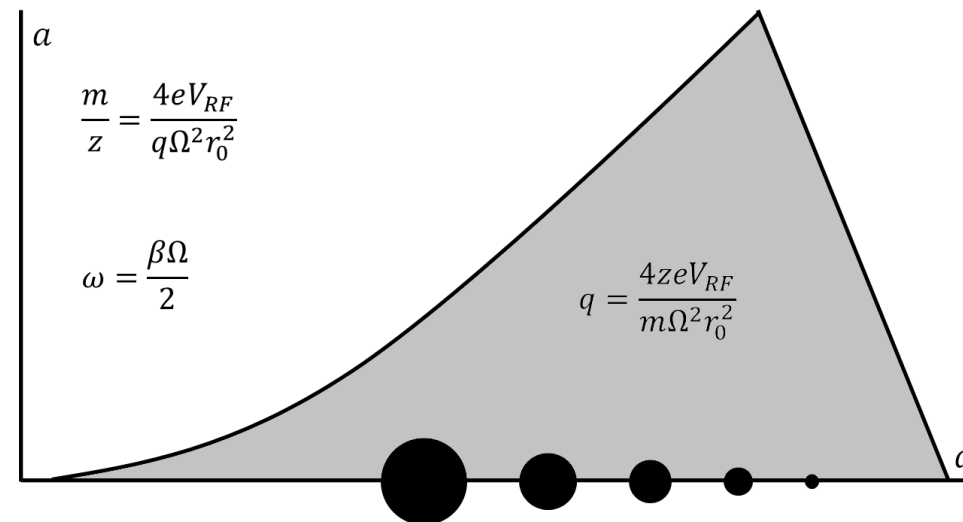
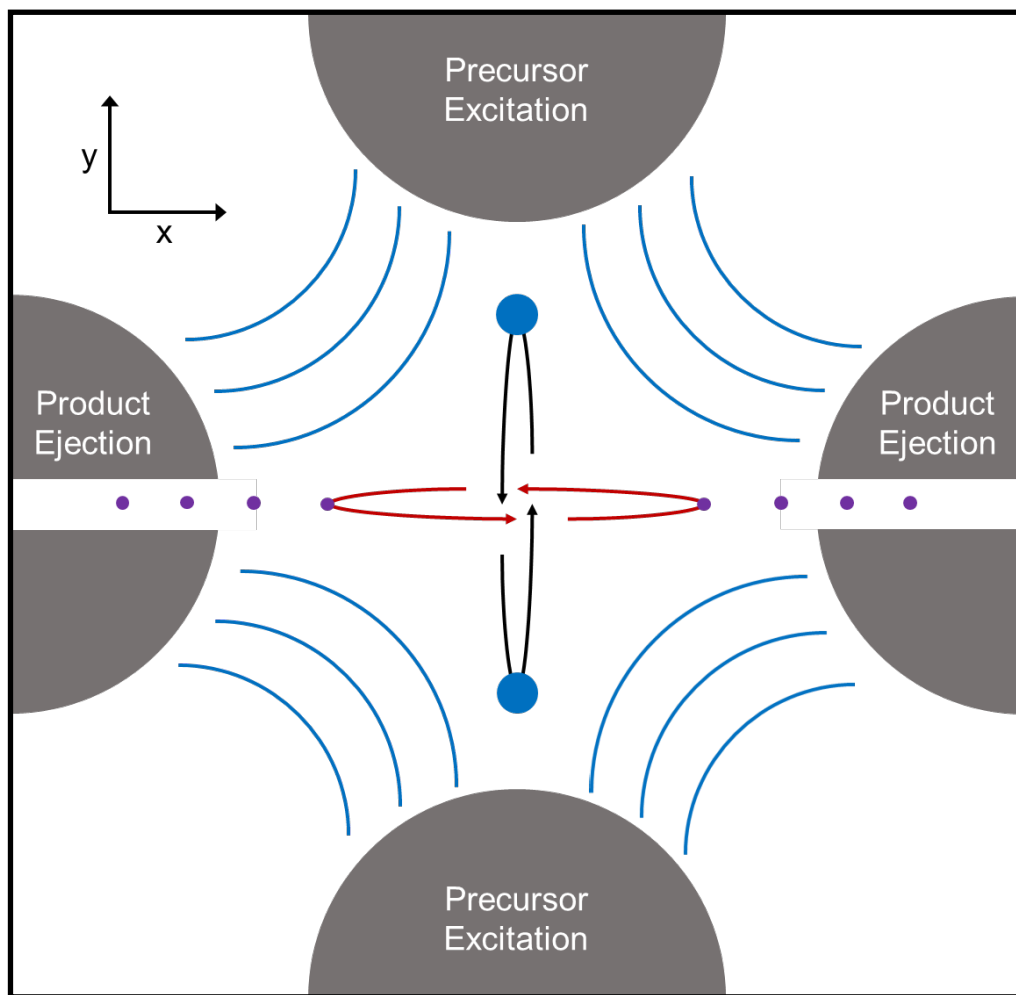


Mass Spectrometry

- nESI
- Modified Thermo LTQ LX
- Supplementary Waveforms
- Linear Quadrupole Ion Trap
- MSSuite Software



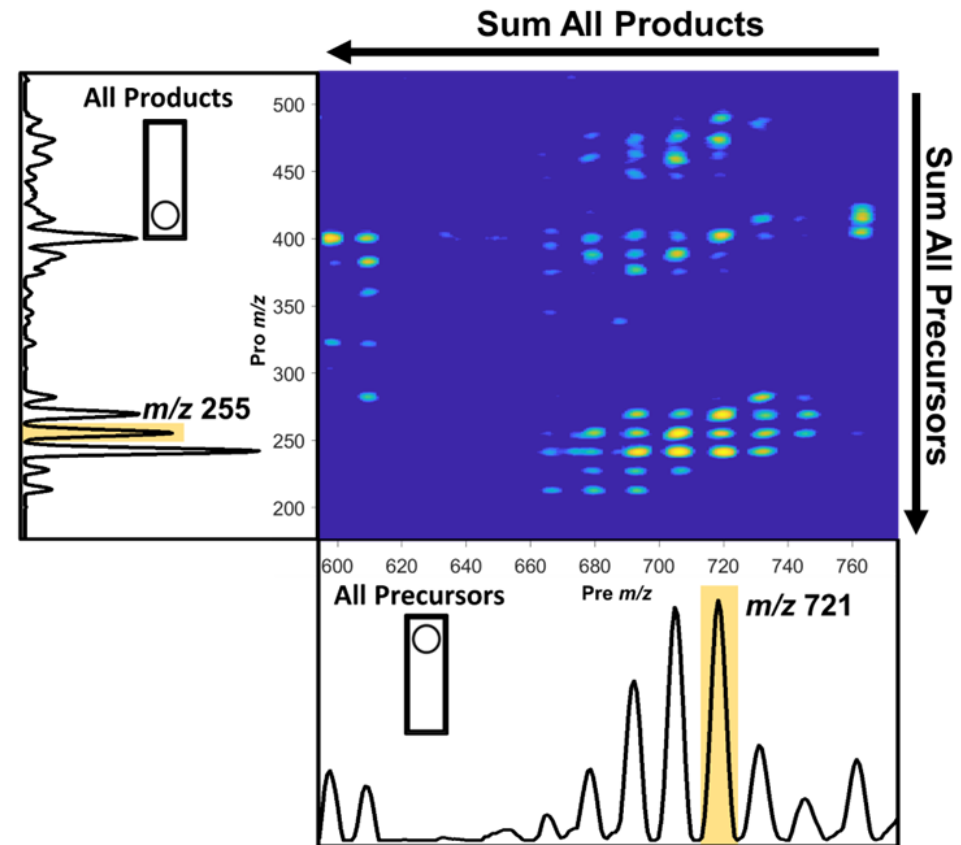
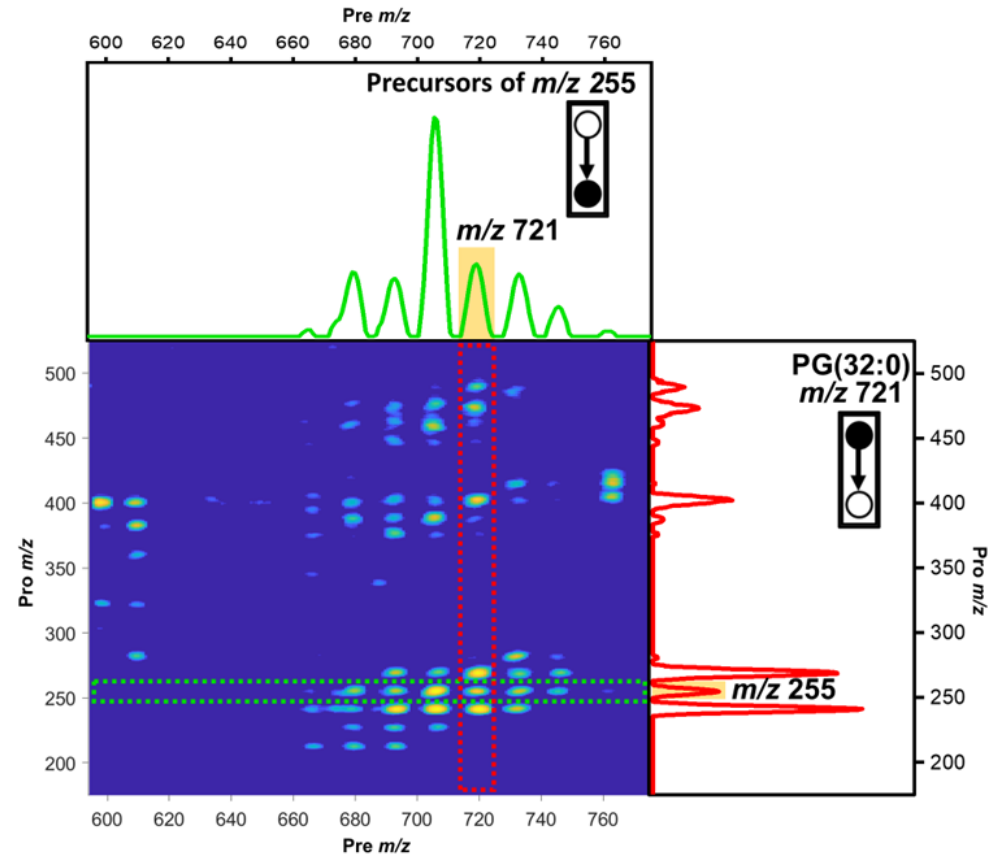
Two-Dimensional Tandem Mass Spectrometry



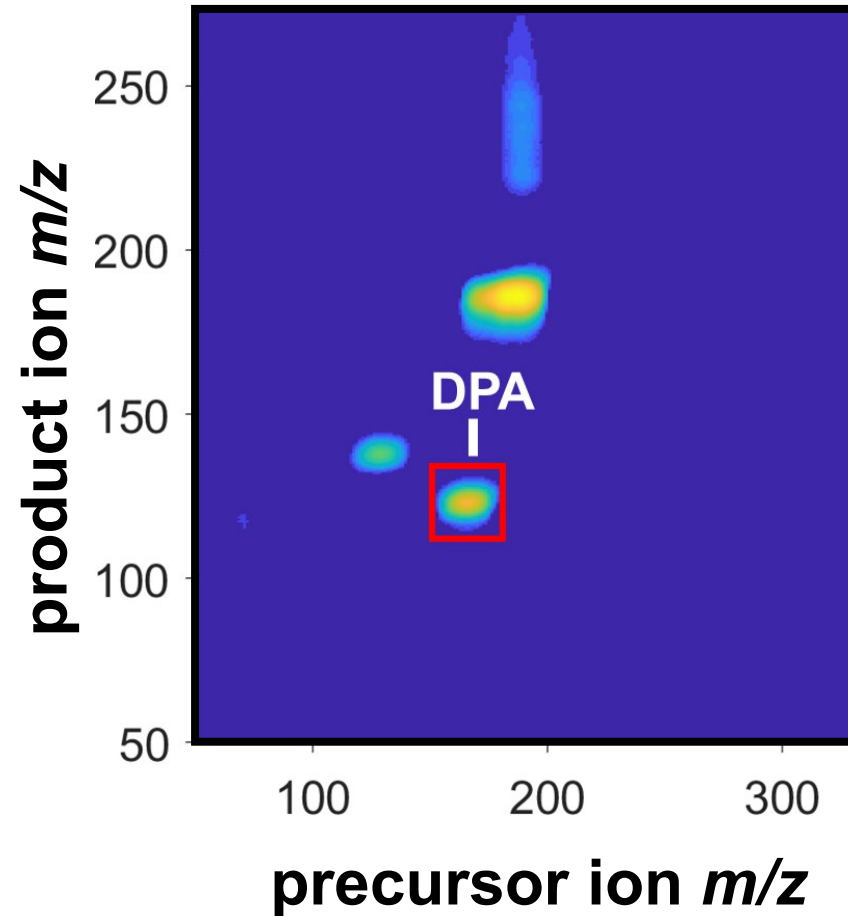
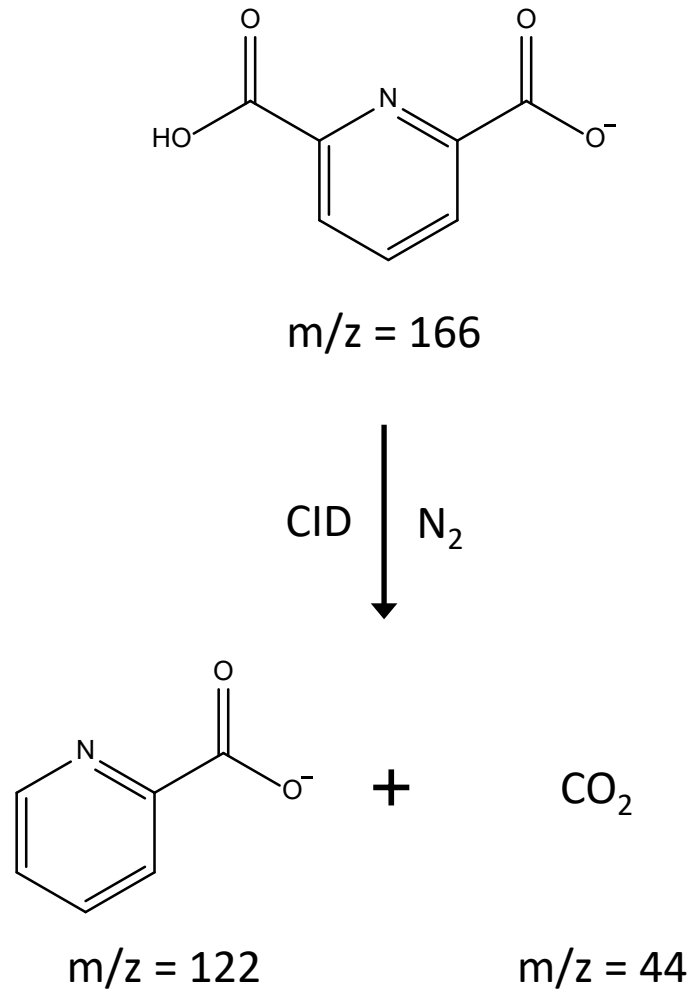
1) Snyder, D. T.; Pulliam, C. J.; Ouyang, Z.; Cooks, R. G. *Anal. Chem.* **2016**, *88*, 2–29, DOI: 10.1021/acs.analchem.5b03070

2) Snyder, D. T.; Cooks, R. G., Single Analyzer Precursor Ion Scans in a Linear Quadrupole Ion Trap Using Orthogonal Double Resonance Excitation. *Journal of the American Society for Mass Spectrometry* 2017

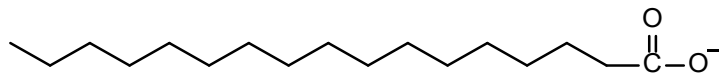
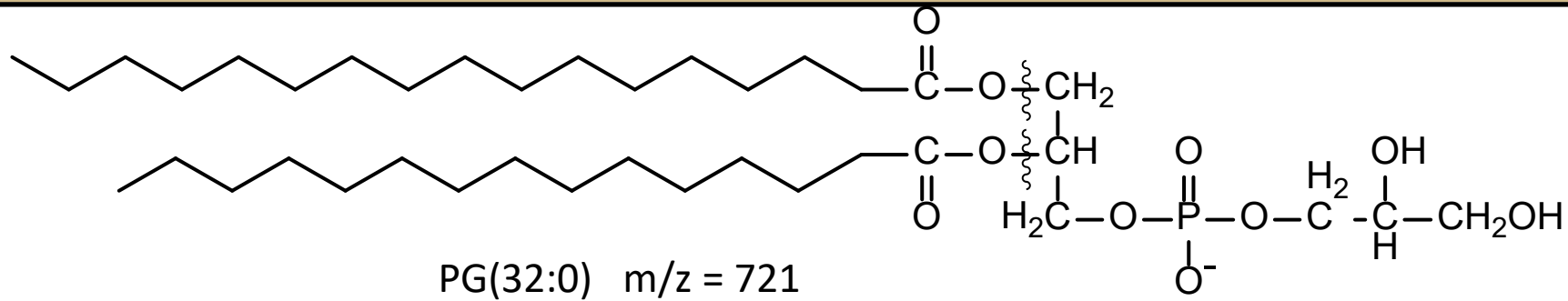
Two-Dimensional Tandem Mass Spectrometry



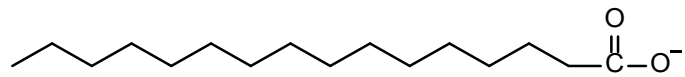
Sporulation Metabolite of *B. subtilis*



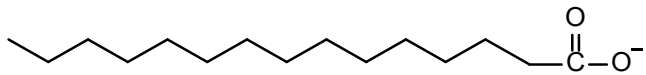
Sporulated Lipids of *B. subtilis*



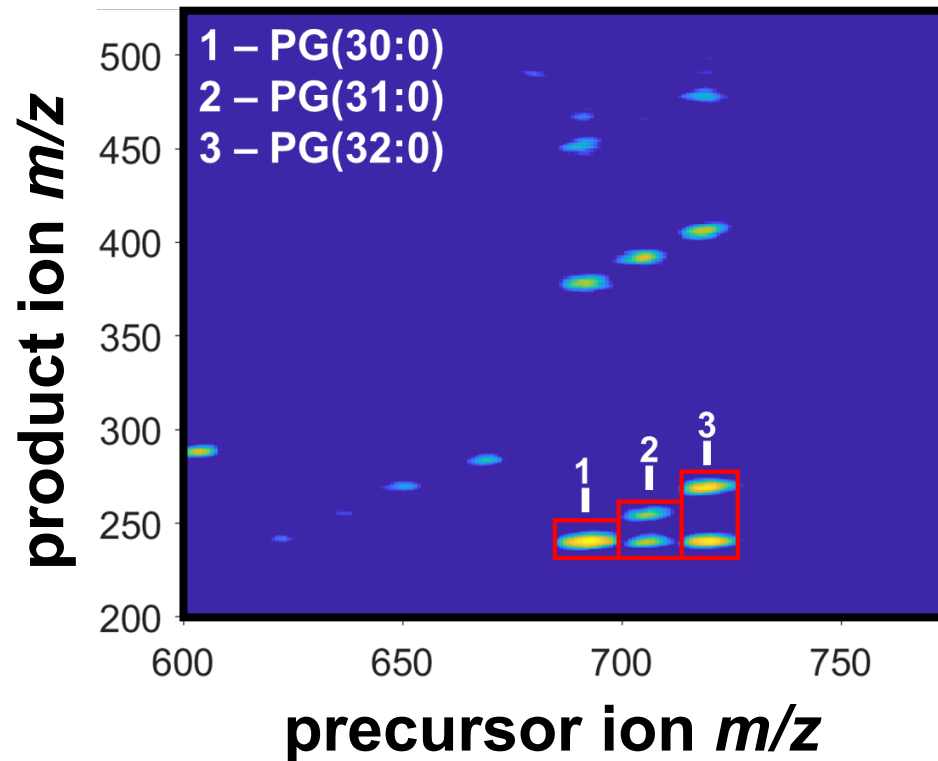
C17:0 $m/z = 269$



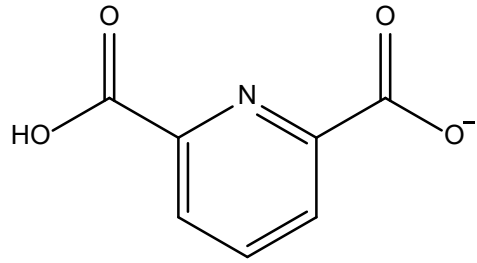
C16:0 $m/z = 255$



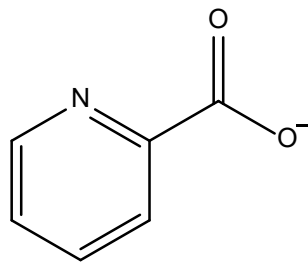
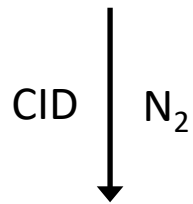
C15:0 $m/z = 241$



Vegetative Metabolite of *B. subtilis*



$m/z = 166$

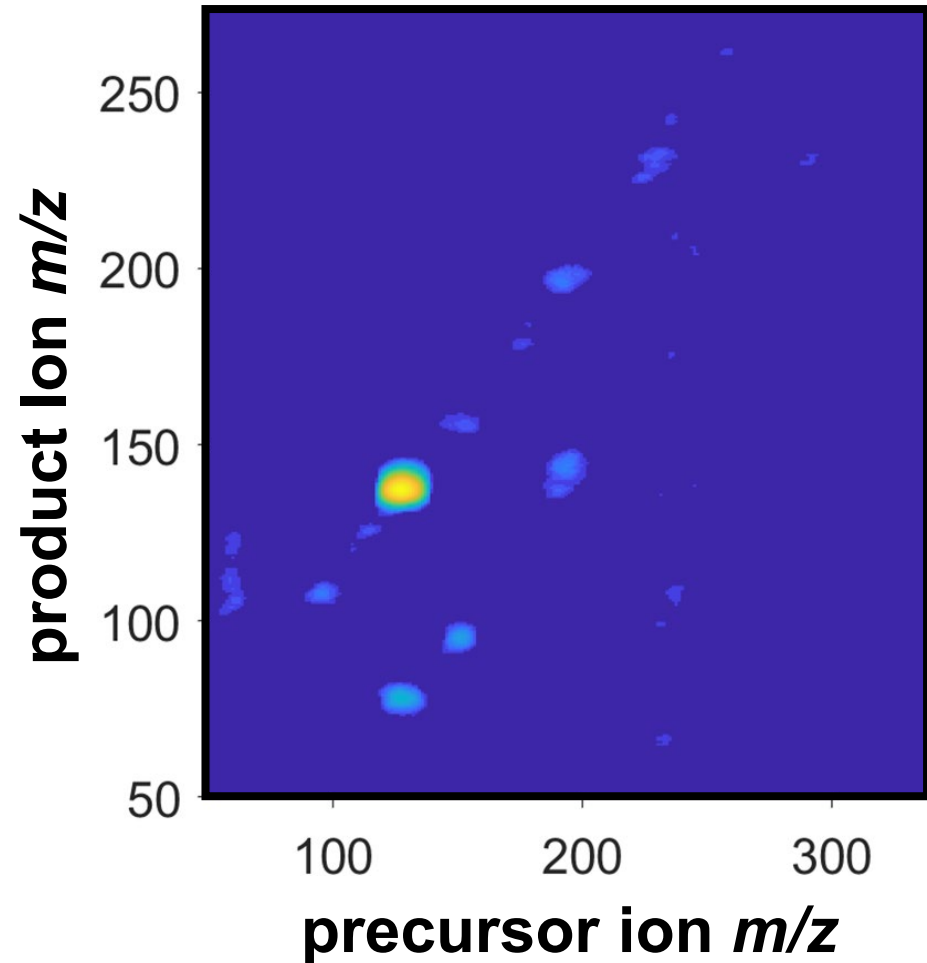


$m/z = 122$

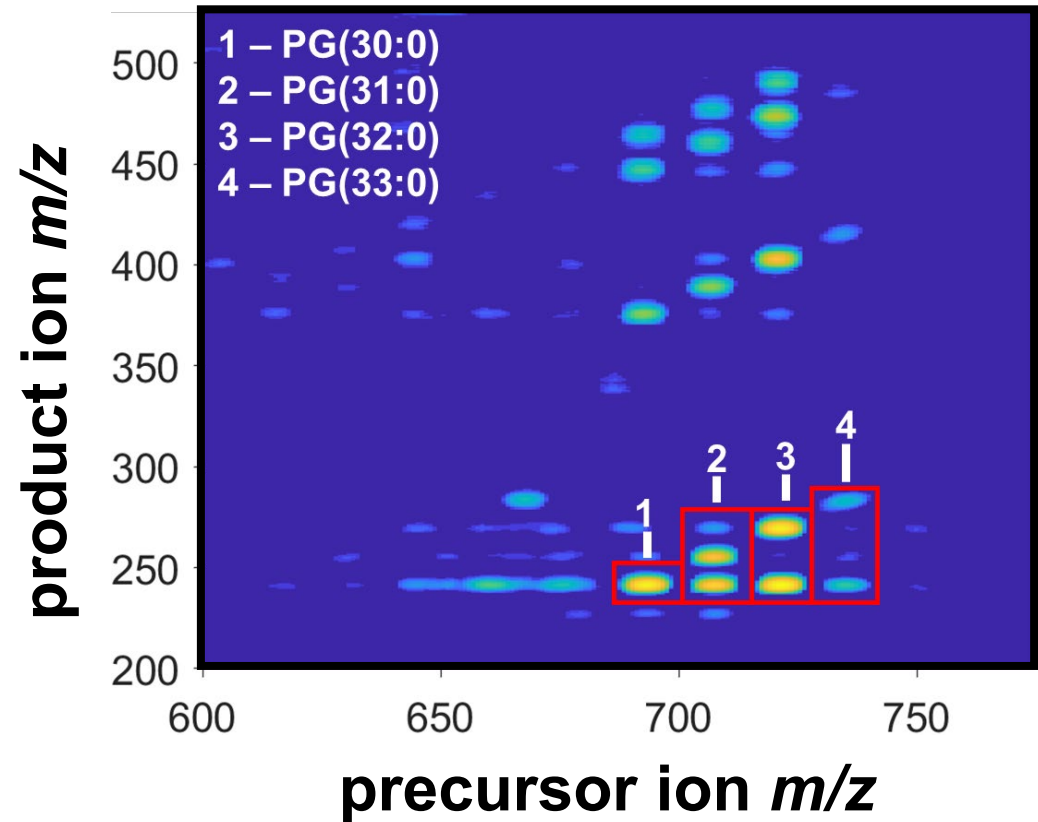
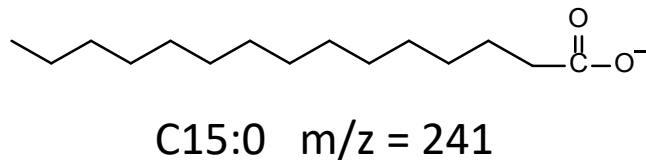
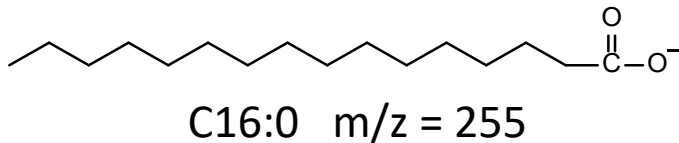
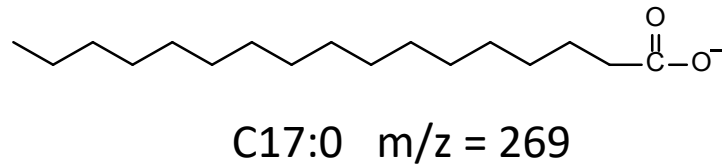
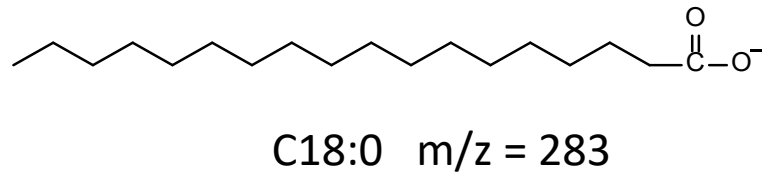
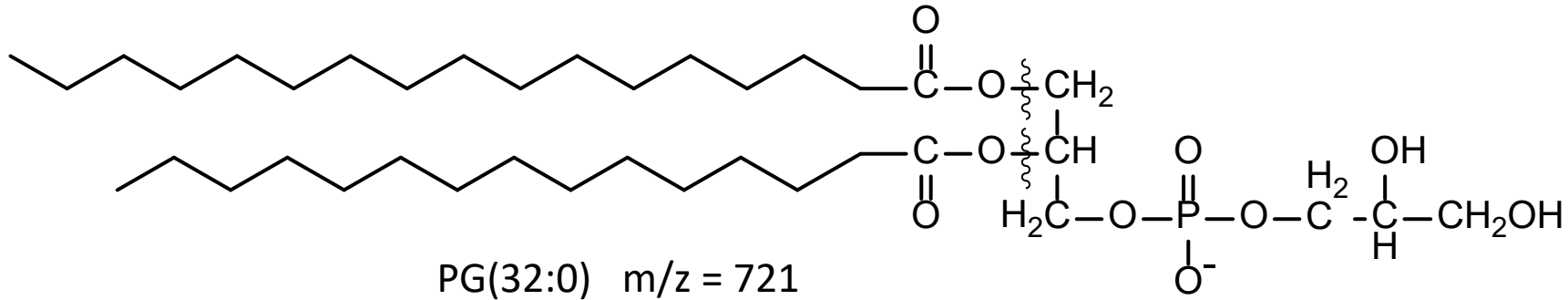
+



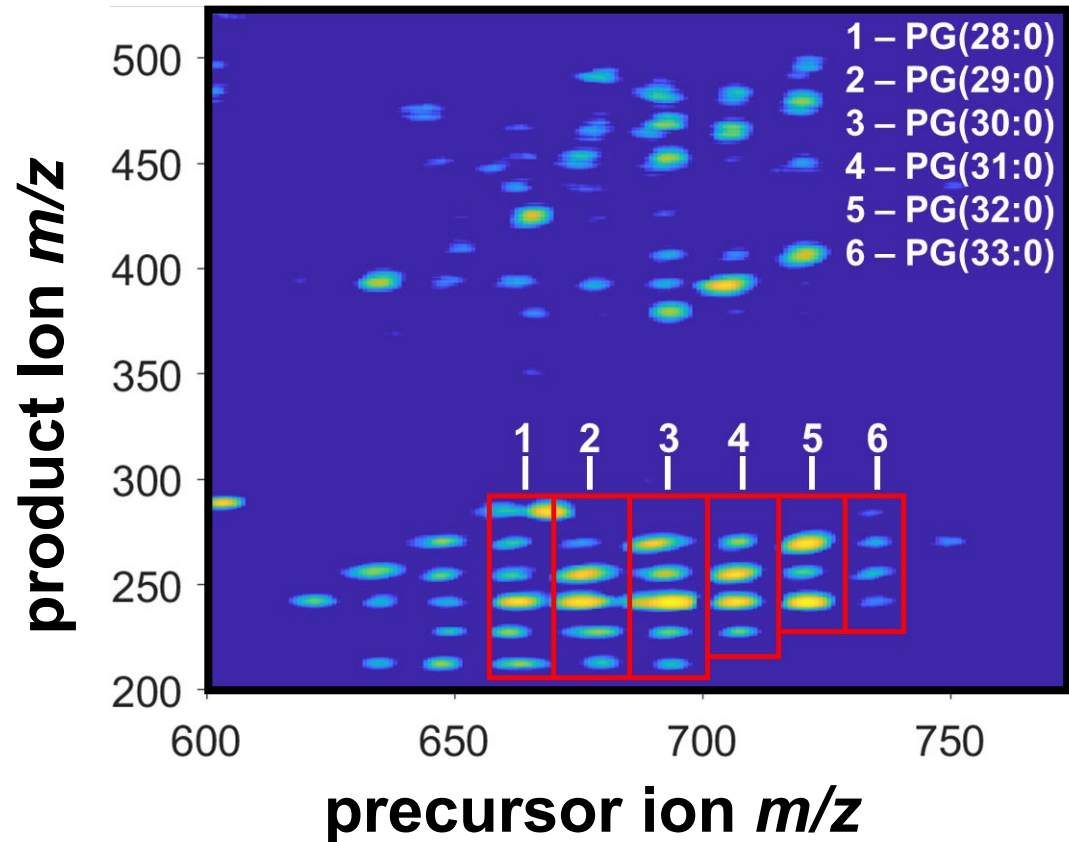
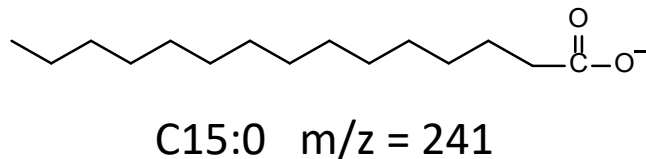
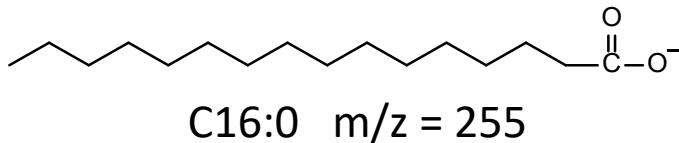
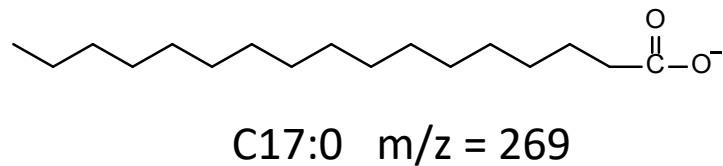
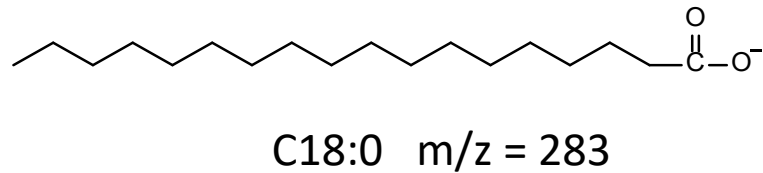
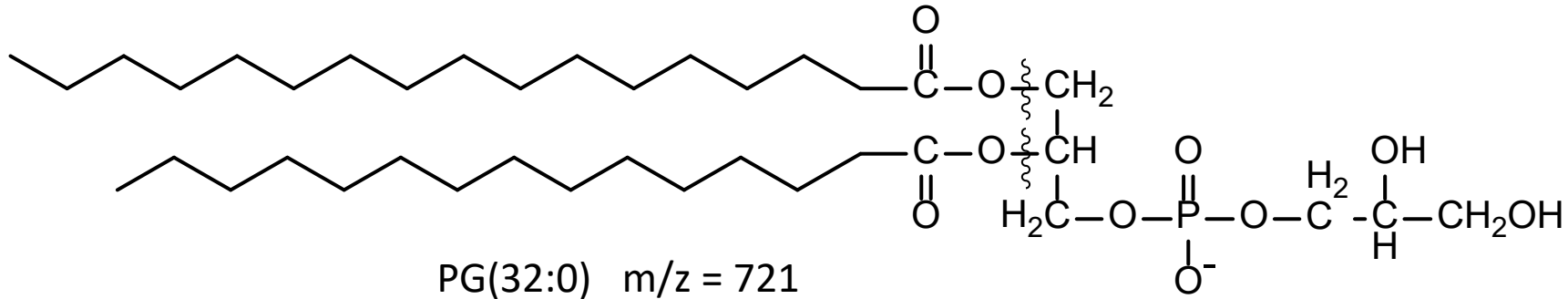
$m/z = 44$



Vegetative Lipids of *B. subtilis*

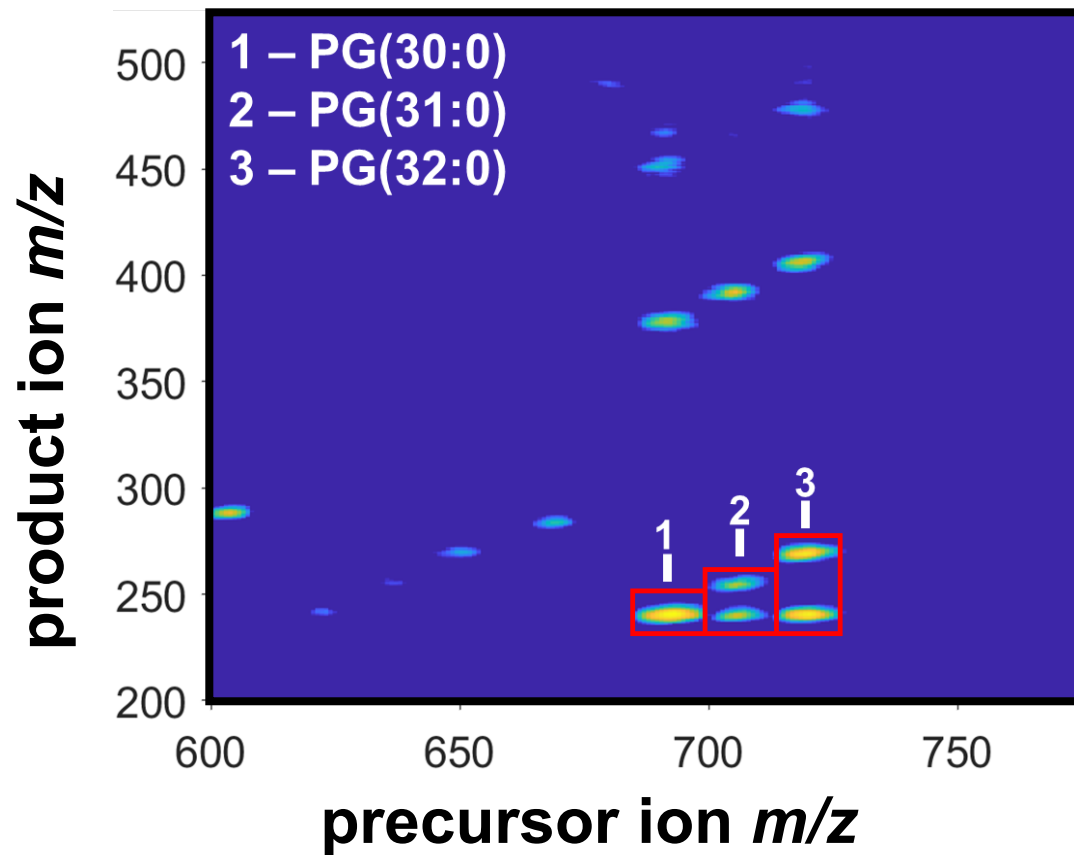


Sporulated Lipids of *B. thurgiensis*

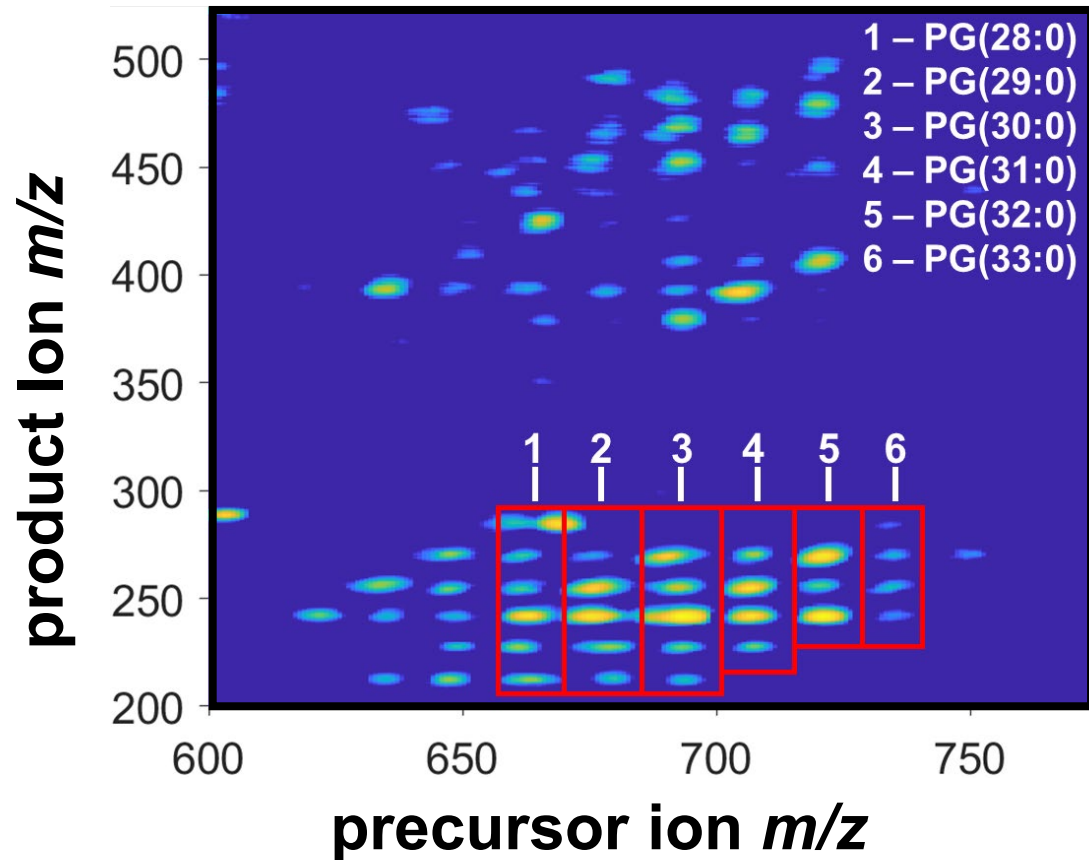


Bacterial Identification

Lipid Region *B. subtilis*



Lipid Region *B. thurgensis*



Opportunities

- Threat Analysis
- Lipidomics
- Lipid Libraries
- Rapid In Situ Analysis
- Miniaturization
- Qualitative Analysis
- Harsh Environments



Acknowledgments

- Thanks to Edwin, Lucas, Eric, and Graham
- Thanks to the rest of Aston Labs
- Funded by the Department of Homeland Security

