Molecular structural analysis of the Gram-positive bacterial membrane - *Enterococcus faecium* and *Staphylococcus aureus*

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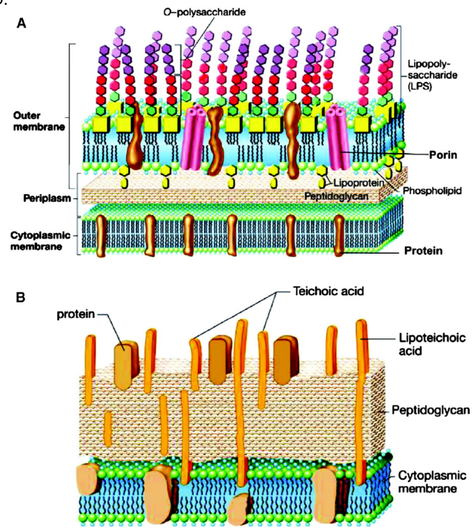
Introduction

Our lab has developed rapid identification method of infectious agents to improve patient outcomes, antimicrobial stewardship, and length of hospital stay [1]. Gram-positive bacteria include a wide variety of important commensals and pathogens. These bacteria normally colonize skin, genital, respiratory, and oral mucosal sites, and activation of the innate immune response by bacteria results in acute inflammation. Gram-positive bacteria are characterized by a single glycerophospholipid membrane enclosed by a thick peptidoglycan cell wall. Lipoteichoic acid (LTA), is a dominant cell wall component. Cardiolipin is also found in the membranes of most bacteria, and is also an important component of the inner mitochondrial membrane in eukaryotes. Here, we have thoroughly investigated structural derivation of Gram-positive bacterial membrane extracts using tandem MS.

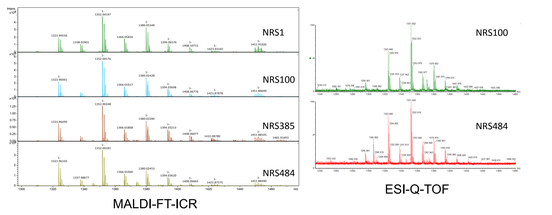
Gram-positive bacteria were grown in lysogenic broth at 37°C overnight. The rapid, hot ammonium isobutyrate micro-extraction protocol [2] and Bligh-Dyer extraction method [3] were used for *Enterococcus faecium* and *Staphylococcus aureus,* respectively. Prior to mass spectrometric analysis, the extracted samples were dissolved in $CHCl_3/CH_3OH$ (2:1 v/v) solution. MALDI experiments were performed on a Bruker Solarix XR 7T FT-ICR mass spectrometer. Bacterial extracts were analyzed in negative ion mode using the matrix norharmane. Quadrupole CID was used for tandem measurements. ESI experiments were performed on a Waters Synapt G2 Q-TOF mass spectrometer. Bacterial extracts were infused at 3uL/min and sensitivity mode was used in the negative ion mode. Tandem MS was carried out using trap CID.



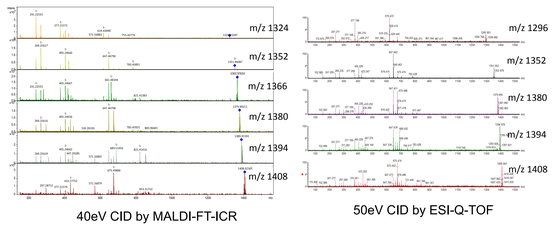
Membranes of (A) Gram-negative bacteria and (B) Gram-positive bacteria. [4]

Results

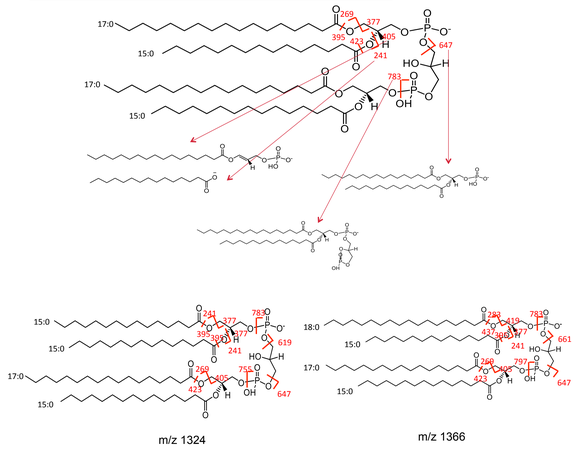
MS1 results of *S. aureus* extracted by hot ammonium isobutyrate micro-extraction protocol [2]



MS2 results of NRS 100 obtained by Q-CID

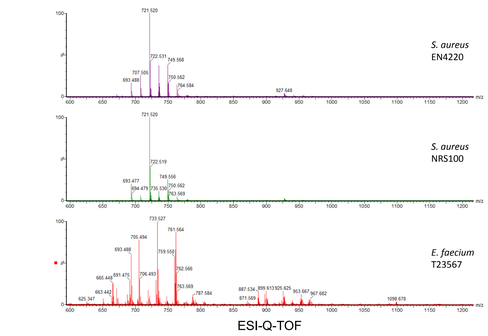


Fragmentation pathways of cardiolipin (m/z 1352)

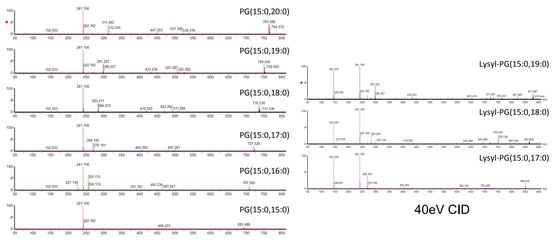


m/z 1380 has (19:0,15:0,17:0,15:0), m/z 1394 has (20:0,15:0,17:0,15:0) and m/z 1408 has (19:0,15:0,19:0,15:0). Ions from *E. faecium* were not abundant enough to have fragmentation.

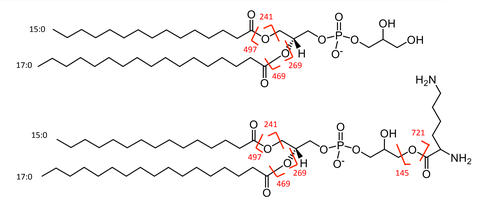
MS1 results of *S. aureus* and *E. Faecium* extracted by Bligh-Dyer protocol [3]



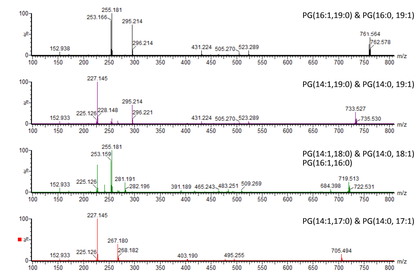
MS2 results of *S. aureus* NRS 100 obtained by Q-CID



Fragmentation of phosphatidylglycerol and lysyl-phosphatidylglycerol



MS2 results of *E. faecium* obtained by 35eV Q-CID



Conclusions

- Hot ammonium isobutyrate extraction protocol extracted mostly cardiolipin ions.
- Bligh-Dyer extraction methods extracted phosphatidyl glycerol which is the major component of membrane.
- *E. faecium* has unsaturated lipid which *S. aureus* has saturated lipid
- Methicillin-resistant Staphylococcus aureus (MRSA, NRS100) and methicillin- sensitive Staphylococcus aureus (MSSA, EN4220) gave similar results.
- MALDI-Q-CID analysis is comparable to that of ESI-Q-CID.

Acknowledgments

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References

1. Leung, L. M., Fondrie, W. E., Doi, Y., Johnson, J. K., Strickland, D. K., Ernst, R. K., and Goodlett, D. R.: Identification of the ESKAPE pathogens by mass spectrometric analysis of microbial membrane glycolipids. *Scientific Reports*, in press
2. Caroff, M.,Karibian, D.: Structure of bacterial lipopolysaccharides. *Carbohydr. Res.* **338,** 2431–2447 (2003)
3. Bligh, E. G., Dyer, W. J.: A Rapid Method of Total Lipid Extraction and Purification. *Can. J. Biochem.Physiol.* **37**, 911-917 (1959)
4. Wang, G., Mishra, B., Epand, R. F., and Epand, R. M.: High-quality 3D structures shine light on antibacterial, anti-biofilm and antiviral activities of human cathelicidin LL-37 and its fragments, *Biochimica et Biophysica Acta*, **1838**, 2160–2172 (2014)

Presentations from Goodlett Lab and Ernst Lab

TOF pm 03:30 Detecting Antibiotic Resistance by MALDI-TOF Analysis of Bacterial Membrane Glycolipids
WOD pm 04:10 Comparison of Quadrupole and Ion Trap Collision Induced Dissociation for Structure Determination of *Francisella Nnovicida* Lipid A variants
WP421 Structure Activity Relationship Elucidation of Pseudomonas Aeruginosa Lipopolysaccharide Variants Associated with Cystic Fibrosis using a Multivaried Mass Spectrometric Approach
WP488 Identification of ESKAPE Pathogens by MALDI-TOF MS Analysis of Microbial Membrane Glycolipids
WP490 Qualitative and Quantitative Analysis of Hemolytic Toxins from Dinoflagellates Specifically Associated with Fish Kills by Mass Spectrometry
WP491 Ultra-Rapid Identification of Bacteria by MALDI-TOF MS
WP589 A SRM/MRM Based Targeted Proteomics Strategy for Quantification of Potential Biomarkers of TKI Sensitivity in EGFR Mutated Lung Adenocarcinoma

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