Ultra-rapid Identification of Bacteria by MALDI-TOF MS

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Introduction & Methods

Conventional methods for classifying and identifying bacterial species relied heavily on biochemical and morphological tests of colonies obtained from cultured agar dishes. These methods may take days or weeks to identify bacteria. Here we present a novel, rapid extraction procedure to identify bacteria by MALDI-TOF MS detection of bacterial glycolipids, such as the lipid A component of LPS from Gram negative bacteria. This new procedure reduces lipid A extraction time from conventionally several days to less than 1.5 hours. The new extraction method works in conjunction with our MALDI-TOF MS based bacterial glycolipid phenotyping approach[1] to allow rapid bacterial identification from a library of containing >75 mass spectral entries.

Bacterial pellets or pure colonies were processed by suspension in 100mM sodium acetate buffer (pH 3.5) for 1 hour incubation at 100°C. During incubation, the glycosidic bond between lipid A and the KDO_2 core sugars of lipopolysaccharide molecules is hydrolyzed liberating lipid A. The mixture is cooled on ice to room temperature and washed with acidified ethanol, 95% ethanol, and 100% ethanol sequentially. The insoluble lipid A is finally extracted and reconstituted in chloroform/methanol/water (3: 1.5: 0.25, v/v/v) mixtures for MALDI-TOF MS analyses. The extraction condition (pH and incubation time) was optimized by using Design of Experiment (DOE). Monophosphate lipid A (MPL) was spiked as an internal standard for quantitative anlysis. Mass spectra were acquired in negative ion reflectron mode on a Bruker Microflex.

New Method vs. Conventional Caroff Method

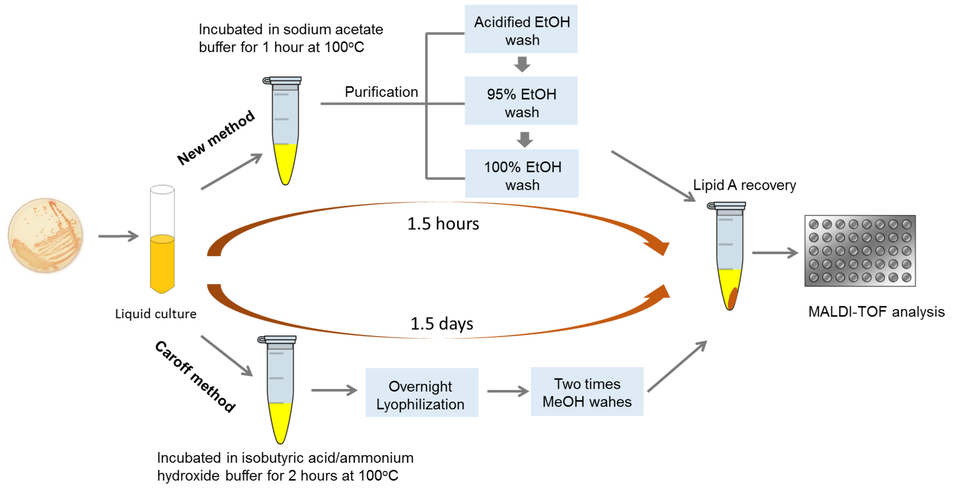

Fig.1. Comparison between the New Method and conventional Caroff method[2].

Design of Experiment Analysis

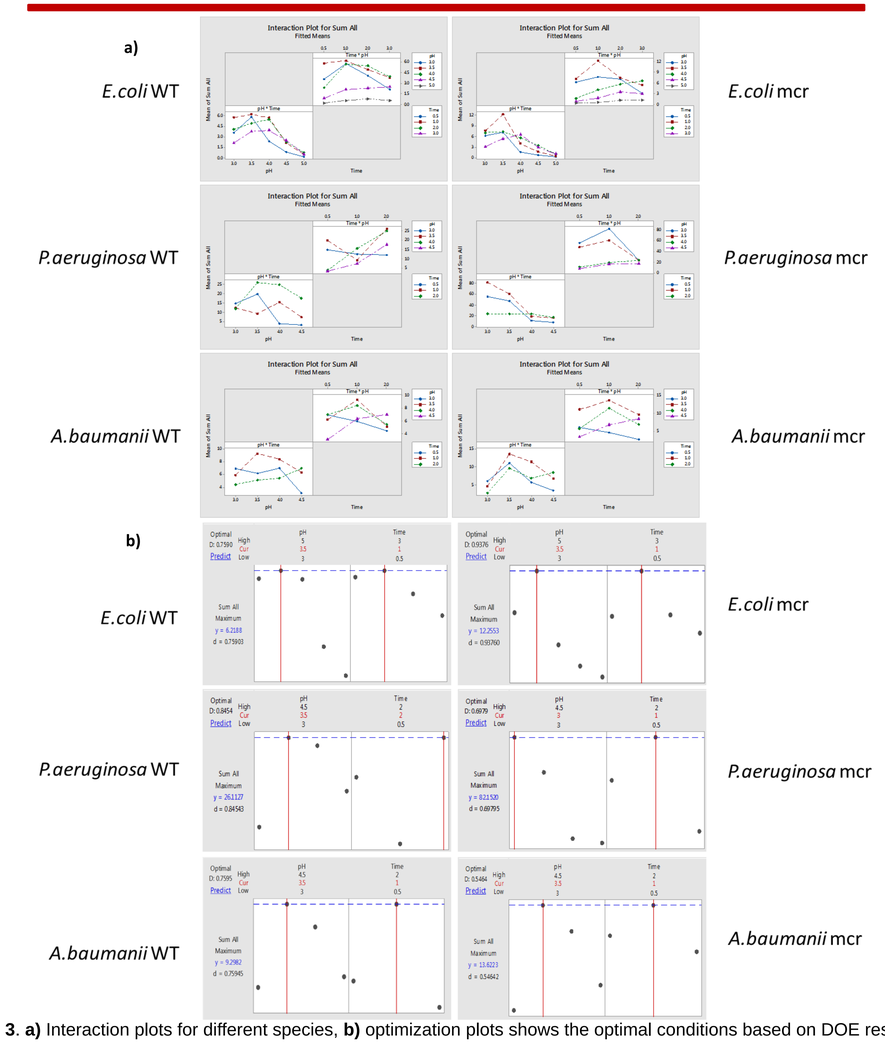

Fig. 3. **a)** Interaction plots for different species, **b)** optimization plots shows the optimal conditions based on DOE results

Mcr Strains Add Phosphoethanolamine on Lipid A

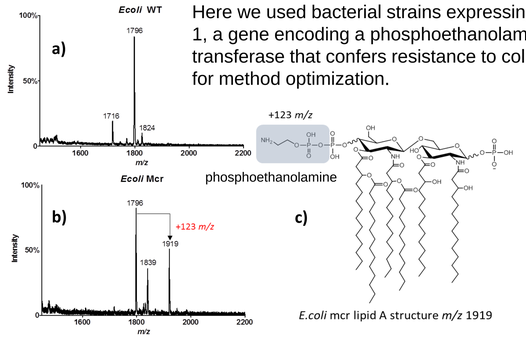

Fig. 2. a) wild type (WT) *E.coli* mass spectrum, lipid A ions are observed at *m/z* 1796, **b)** mcr *E.coli* mass spectrum, modified lipid A ions are observed at *m/z* 1919. **c)** lipid A structure of ion *m/z* 1919.

Here we used bacterial strains expressing mcr-1, a gene encoding a phosphoethanolamine transferase that confers resistance to colistin, for method optimization.

Bacterial Species
E. coli WT
E. coli +mcr
Pseudomonas aeruginosa WT
Pseudomonas aeruginosa +mcr
Acinetobacter baumannii WT
Acinetobacter baumannii +mcr

Table 1. Bacterial species used in DOE study

Design of Experiment for Method Optimization

The extraction condition was optimized by using DOE. Two variables, time (0.5 to 2 h) and pH (3.0 to 5.0) were considered the most important parameters for optimization Optimal conditions were selected based on the sum of ratio of signal intensities of lipid A signature ions relative to a spiked MPL standard.

Factor	Levels	Level Values				
pH	5	3.0	3.5	4.0	4.5	5.0*
Time (hour)	4	0.5	1.0	2.0	3.0*	

Table 2. Values of pH and incubation time for DOE

Conclusion & Discussion

Species	WT	Mcr
E.coli	pH 3.5, 1.0 h	pH 3.5, 1.0 h
P.aeruginosa	pH 3.5, 2.0 h	pH 3.0, 1.0 h
A.baumanii	pH 3.5, 1.0 h	pH 3.5, 1.0 h

Table 3. Summary of DOE results for different bacterial species

- A new lipid A extraction method has been developed that greatly reduces the extraction time from 1.5 days to 1.5 hours.
- DOE results (**Table 3**) show that for most species, condition of pH 3.5 and 1 hour incubation can extract the most lipid A molecules.
- It is highly recommended that the proper pH and incubation time are optimized for each bacterium.
- New method successfully generates comparable lipid A mass spectra as the widely used “Caroff” method.
- This new method can be incorporated into our glycolipid MS library approach[1] for rapid bacterial identification.

New Method Generates Comparable Lipid A Mass Spectra

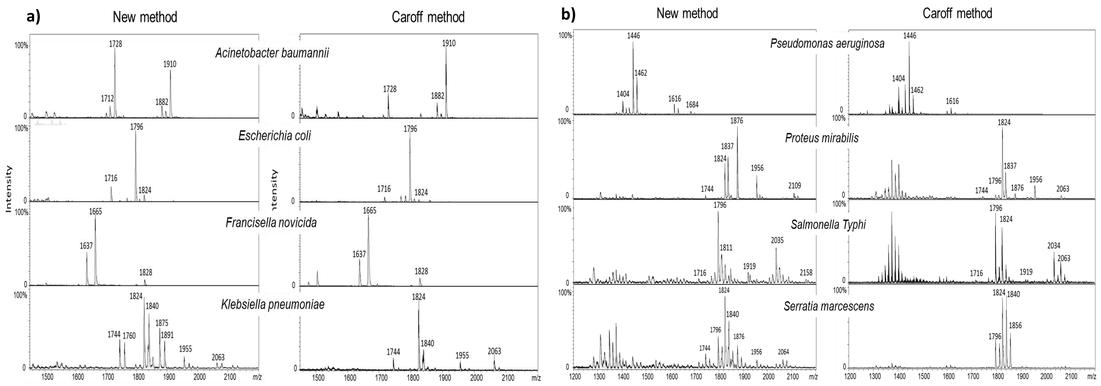

Fig. 4. **a)** and **b)** show lipid A mass spectra of eight different bacterial species using the new method and the Caroff method for lipid A extraction. New method generates highly similar mass spectra compared to the spectra from lipid A library[1] that were produced by the Caroff method[2].

References:

1. Lisa Leung *et. al*. *Sci. Rep.* 2017, In press
2. El Hamidi, Asmaa, *et al. J. Lip. Res.* 46.8 (2005): 1773-1778.

Acknowledgement:

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Oral:

TOF pm 03:30: Detecting Antibiotic Resistance by MALDI-TOF Analysis of Bacterial Membrane Glycolipids. **William Fondrie**

WOD pm 04:10: Comparison of Quadrupole and Ion Trap Collision Induced Dissociation for Structure Determination of Francisella Novicida Lipid A Variants. **David Goodlett**

Poster:

WP409: Molecular Structural Analysis of the Gram-positive Bacterial Membrane – Enterococcus faecium and Staphylococcus aureus. **Sung Hwan Yoon**

WP421: Structure Activity Relationship Elucidation of Pseudomonas aeruginosa Lipopolysaccharide Variants Associated with Cystic Fibrosis using a Multivaried Mass Spectrometric Approach. **Mohsin Khan**

WP488: Identification of ESKAPE Pathogens by MALDI-TOF MS Analysis of Microbial Membrane Glycolipids. **Lisa Leung**

WP490: Qualitative and Quantitative Analysis of Hemolytic Toxins from Dinoflagellates Specifically Associated with Fish Kills by Mass Spectrometry. **Benjamin Oyler**

WP589: A SRM/MRM Based Targeted Proteomics Strategy for Quantification of Potential Biomarkers of TKI Sensitivity in EGFR Mutated Lung Adenocarcinoma. **Shivangi Awasthi**