Modified MALDI MS Fatty Acid Profiling for Bacterial Identification

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Overview

•Fatty acid profiles of bacteria were analyzed using CaO as a matrix replacement, which has been shown to cleave fatty acids *in situ* from lipids during ionization in a MALDI MS instrument.

•Principle components analysis and cross-validation of bacterial fatty acid spectra showed distinct groupings and good correlation.

•MOLI MS could provide clinicians with a fast, reliable method for bacterial ID using the same instrumentation increasingly used for protein-based bacterial diagnostic identification.

Introduction

•Early clinical bacterial identification is crucial for effective treatment.

•Bacterial ID based on fatty acid (FA) profiles was previously reported.[1]

• Metal oxide laser ionization mass spectrometry (MOLI MS) is a new technique using CaO as a matrix replacement to cleave lipids and effectively ionize a sample without producing interfering peaks in the low mass region of the mass spectrum.[2]

• This extends the usable range of matrix-assisted laser desorption/ionization (MALDI) to small molecules.

•FA profiles of ten bacterial species were analyzed by MOLI MS and principal components analysis (PCA) to provide a 3-D visualization of the data.

•Cross-validation was used for bacterial ID and model correlation.

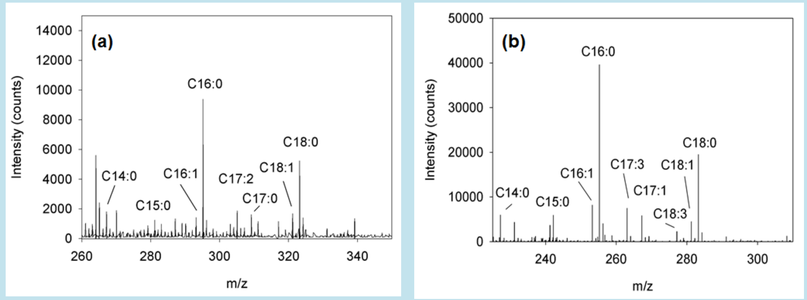

Figure 1: MOLI MS spectra of *E. coli* fatty acids in positive-ion (a) and negative-ion (b) modes

Methods

•Bacteria were cultured overnight on either brain-heart infusion or Lauria Bertani media.

•Individual colonies were suspended in 200 µL of phosphate buffered saline, pH 7.4 (PBS).

•Lipids were extracted by addition of 200 µL of 33/66 v/v % methanol/chloroform and vortexed for 30 seconds.

•100 mg of CaO was mixed with 1 mL of n-hexane; 1 µL of the resulting slurry was spotted on a MALDI plate.

•One µL of 0.15M HCl in methanol was added to activate CaO

•Measurements were taken on a JEOL JMS-S3000 SpiralTOF mass spectrometer

- 349 nm Nd:YLF laser at 250 Hz
- Laser fluence at 55%
- Resolution 37,000
- 150 shots/spectrum in both positive- and negative-ion mode

•PCA and cross-validation were performed on FA peaks listed in Table 1 with the R software package.

Table 1. Fatty Acids used in Principal Component Analysis

Fatty Acid	Mass	$[M-H+Ca]^+$	$[M-H]^-$
C14:0	228	267	227
C15:0	242	281	241
C16:1	254	293	253
C16:0	256	295	255
C17:1	268	307	267
C17:0	270	309	269
C18:1	282	321	281
C18:0	284	323	283
C19:0	298	337	297
C20:0	312	351	311
C21:0	326	365	325

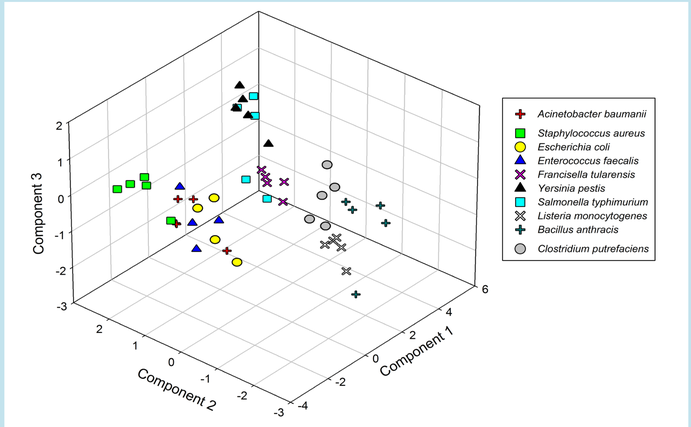

Figure 2: PCA plot of fatty acid spectra collected in positive-ion mode.

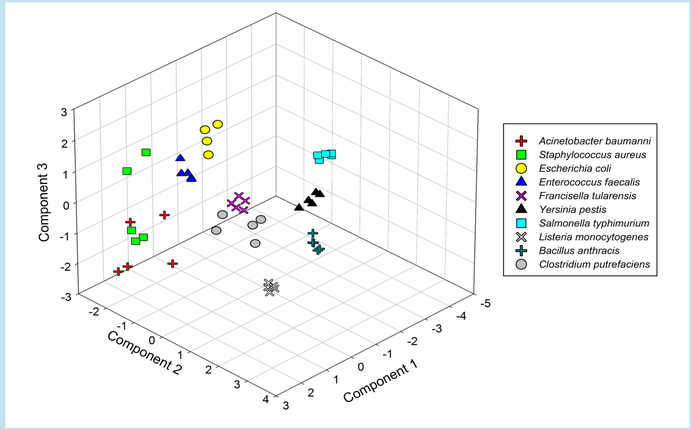

Figure 3: PCA plot of fatty acid spectra collected in negative-ion mode.

Results

•Distinct grouping was observed in both positive-ion (Figure 1) and negative-ion (Figure 2) mode.

•However, negative-ion data showed the best clustering of bacterial species.

•*Acinetobacter baumanii* and *Staphylococcus aureus* could not be adequately resolved in either positive- or negative-ion. Changing 3-D perspective and changing the principal components on the plot did not resolve this issue.

•Cross-validation of the positive-ion data resulted in one false positive and 15 false negatives out of 470 measurements.

•Similar analysis of the negative-ion data produced one false positive, but only 4 false negatives out of 490 measurements.

Conclusions

MOLI MS coupled with PCA for analysis of FA peaks in bacteria showed distinct groupings for each species. Negative-ion mode showed much better separation of species. Both positive- and negative-ion modes had excellent cross-validation records, demonstrating the effectiveness of the model. MOLI MS can provide clinicians a rapid, reproducible, and cost-effective bacterial ID diagnostic tool.

References

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2.McAlpin, C.R., Voorhees, K.J., Corpuz, A.R., Richards, R.M.: Analysis of lipids by metal oxide laser mass spectrometry. *Anal Chem.* **84**, 7677 (2012).

3.Voorhees, K.J., Jensen, K.R., McAlpin, C.R., Rees, J.C., Cody, R., Ubukata, M., Cox, C.R. Modified MALDI MS fatty acid profiling for bacterial identification. *J Mass Spectrom.* (Accepted)