

Characterization of bile components associated with gall bladder infection by *Listeria monocytogenes*.

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Overview

- Comparative study of *Listeria monocytogenes* in animal models: uninfected healthy, infected healthy, and sick with generalized infection
- Use of alignment and differential analysis software (SIEVE) revealed differences difficult to spot otherwise
- Unique, up-, or down-regulated features found in each type of sample are presented
- High resolution MS of precursor and fragment ions assists in further characterizing compounds of interest

Introduction

Listeria monocytogenes, a bacterium, causes severe systemic illness, especially in pregnant women. *L. monocytogenes* was engineered for bioluminescence, and sequential images of infection in individual live mice were recorded by detecting the light emission from the bacteria. These studies revealed that *L. monocytogenes* grows in the lumen of the gall bladder (Fig. 2), surviving on pure bile, which has not been described for any other organism in an animal model¹. We are interested in how the bacteria survive and grow in the pure bile of the gall bladder lumen.

In the present study, the focus is on differential analysis of samples from infected and uninfected animals as well as animals showing signs of illness not related to the gall bladder infection. LC-MS and LC-MS/MS techniques previously developed in our laboratory are applied to these bile samples.

FIGURE 1. LC-MS analysis of bile – positive (A) and negative (B) full scan modes⁴. Fragmentation studies show that the majority of the sample components can be classified either as bile acid derivatives² or as phosphatidylcholines³.

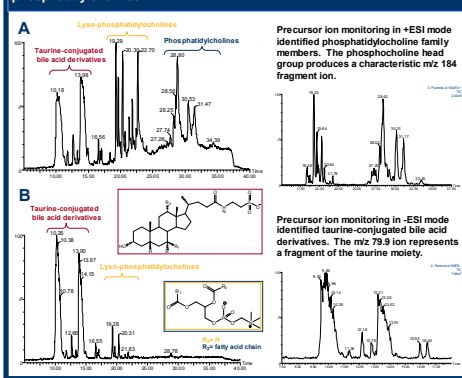
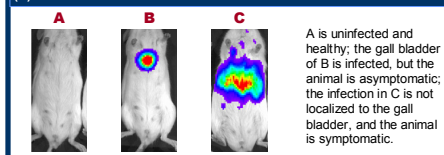


FIGURE 2. *In vivo* bioluminescence imaging. *L. monocytogenes* can replicate extracellularly in the gall bladder. Intravenously infected mice display light emission from the bacteria. Bile samples from uninfected (A), infected healthy (B) and generally infected sick animal (C) have been studied.



A is uninfected and healthy, the gall bladder of B is infected, but the animal is asymptomatic; the infection in C is not localized to the gall bladder, and the animal is symptomatic.

Methods

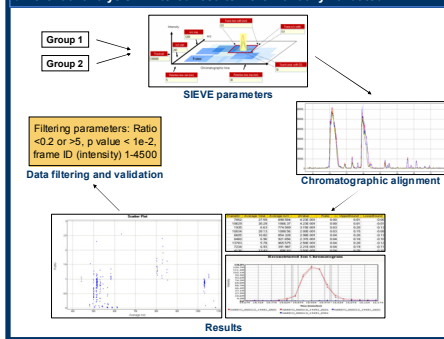
Sample preparation: Female CD1 mice were intravenously infected with bioluminescent *L. monocytogenes*.

Bioluminescence images were taken daily to monitor gall bladder colonization (Fig. 2). The animals were sacrificed and gall bladder bile samples were obtained and diluted with four volumes of isopropanol. Samples were further diluted with methanol prior to LC-MS analysis.

Instrumentation: Analysis was carried out by ESI LC-MS/MS using an Agilent 1100 HPLC and Micromass Quattro Premier triple quadrupole MS. HPLC conditions: Atlantis T3 C18 100 x 2.1 mm column, 3 μm particle size (Waters Corp.); gradient 50-99% B in 20 min, hold at 99% for 14 min, total run time 40 min; A: 2 mM ammonium formate in water, B: 1 mM ammonium formate in methanol; 250 μl/min flow rate. High resolution measurements were acquired on a SpectraSystem P4000 HPLC and Micromass Q-ToF API MS, using identical chromatographic conditions.

Data analysis: SIEVE 1.0 (Thermo Fisher Scientific) was used for differential analysis of samples.

SCHEME 1. SIEVE workflow. LC-MS analyses are compared by selecting appropriate SIEVE parameters to define features, aligning the chromatograms, and generating extracted ion chromatograms for differential analysis. Filtered results were manually validated.



Results

FIGURE 3. Infected vs. uninfected. Two technical replicates for each sample are shown. More than 30 compounds of interest have been found; over 25 of them are unique to the healthy animal, and 5 are present only in the infected bile.

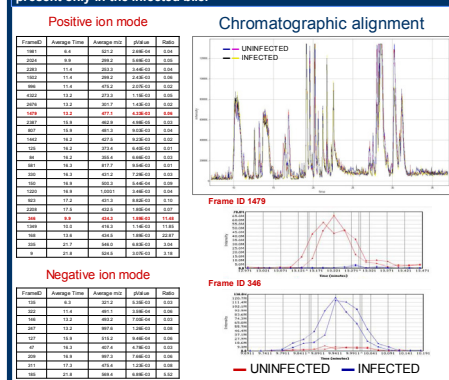


FIGURE 4. Infected healthy vs. generally infected sick. Comparison of healthy infected, and sick with delocalized infection, examines changes related to the state of health. Differences could be due to numerous factors, including dehydration or liver pathology. Over 20 compounds unique or up-regulated in the healthy animal have been found. Lyso-PCs are markedly depleted in the sick animal. Only a small portion of the differences are listed in these tables.

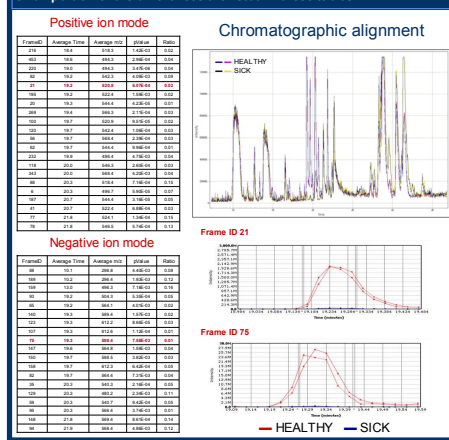
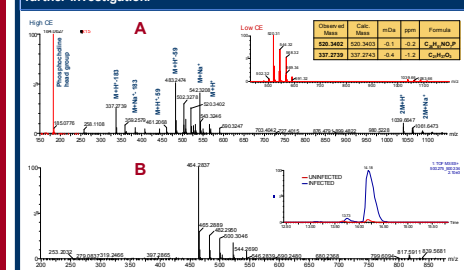


FIGURE 5. LC-MS accurate mass measurements. Example A (RT=19.2 min) is prominent in uninfected healthy animal only, and is consistent with identification as 18:2 lyso-phosphatidylcholine; characteristic fragments are labeled. Example B (RT=14.2 min) increases dramatically in infected healthy animal, and is undergoing further investigation.



Conclusions

- Components unique to each type of sample have been observed
- Findings distinctive to healthy animals may indicate nutrients on which bacteria grow
- Findings distinctive to infected animals may be a sign of bacterial products excreted to the gall bladder lumen during infection
- Use of differential analysis software revealed intensity variations in ions difficult to detect beneath highly abundant common bile components
- HRMS measurements and fragmentation studies supported further identification of compounds of interest

Future work

- Optimize bacterial growth conditions in *ex vivo* inoculated bile to enable further studies on bile components which are depleted by the bacteria
- Continue characterization of compounds of interest found in these studies
- Broaden studies to include human bile

References

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Acknowledgements

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This poster may be downloaded from the Stanford University Mass Spectrometry website at <http://mass-spec.stanford.edu/Publications.html>

