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Comprehensive analysis of metabolites and biomarkers in lung using MALDI-MSI

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Background

Lung injury is often complex, multi-faceted and heterogeneous[1]. For example, radiation-induced lung injury (RILI), resulting from accidental exposure to high-dose radiation or thoracic radiotherapy, includes breakdown of airway epithelium, cellular injury, and inflammatory cell infiltration[2]. RILI shares characteristics with other disease states, such as Chronic Obstructive Pulmonary Disease (COPD), Cystic Fibrosis (CF), and Idiopathic Pulmonary Fibrosis (IPF). Lack of diversity in the lung microbial community has been associated with disease progression for COPD, CF, and IPF. Recent studies have shown metabolites, both endogenous and bacterial, can give unique insight into mechanisms of injury and have the potential to serve as biomarkers[3]. Here, we describe MALDI-MSI methodology to aid in the interrogation of lung metabolites.

Objectives

1. **Interrogate bacterial metabolites in *Pseudomonas aeriginosa* and *Staphylococcus aureus* mono- and co-cultures.**

2. **Repeat our experimental design using a lung infection model.**

3. **Translate MALDI-MSI workflow to DESI-MSI.**

Mass Spectrometry

Mass Spectrometry Methods

Matrix-assisted laser desorption/ionization (MALDI) – mass spectrometry imaging (MSI) was conducted using a Bruker 12T Solarix FT-ICR mass spectrometer. Bacterial Cultures were analyzed at 45% laser power with a minimum laser diameter, 1000 shot steps, and 200 µm raster width in positive ion mode. Lung tissue was imaged using at 30% laser power with a minimum laser diameter, 200 shot steps, and a 60 µm raster width in positive ion mode.

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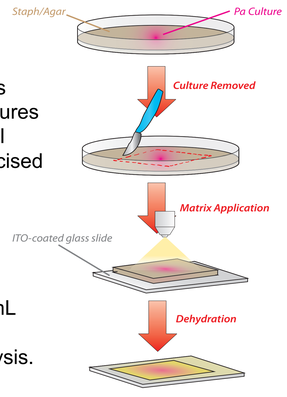
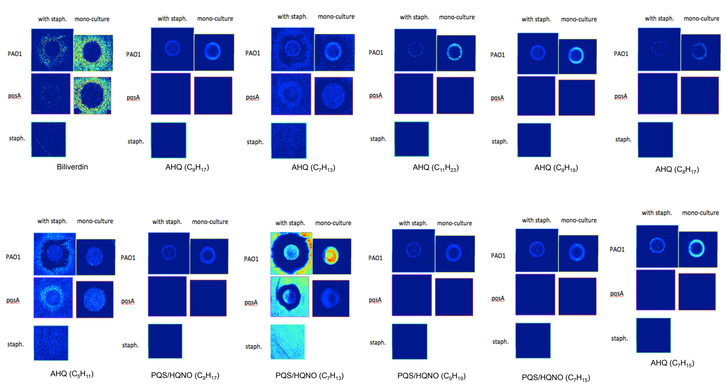
Bacterial Culture Model

Bacterial Culture Model Methods

PAO1 and pqsA strains of *Pseudomonas aeriginosa* were cultured on agar for 24 hours with and without *Staphylococcus aureus*. Cultures were excised from petri dishes using a scalpel and mounted on ITO-coated glass slides. Excised cultures were then coated with 8 passes of 30 mg/mL 1:1 DHB:CHCA with 0.1% TFA in a biosafety hood using an airbrush. Coated-cultures were dried overnight in a 37°C oven before 3 days of desiccation. Dried cultures were then coated a second time with 10 mg/mL 1:1 DHB:CHCA with 0.1% TFA using an HTX Imaging TM-Sprayer before MALDI-MSI analysis.



Bacterial Culture Model Results



MALDI-MSI analysis yields several detectable alkyl quinolone (AQ) metabolites secreted from *Pseudomonas,* while one PQS/AQNO (C_7H_{13}) appears to be secreted from *Staph.* A significant decrease in AQ intensity was observed in pqsA mutants, as these mutants exhibit reduced 4-quinolone synthesis[4]. In addition, biliverdin production was observed PAO1 and pqsA cultures, indicating heme catabolism.

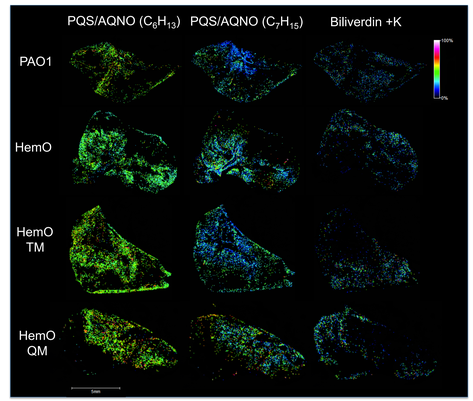
Bacterial Culture Results

Lung Infection Model Methods

Lung tissue from mouse infection models was screened for metabolites of both endogenous and bacterial origin. Mice were infected with PAO1, HemO, HemO TM, or HemO QM *Pseudomonas aeriginosa* via inhalation. Accurate mass isotope matching was used for identification via SCiLS Lab and HDI software. MSI data was searched against a variety of databases including LipidMaps, the Human Metabolome Database (HMDB) and the Pseudomonas aeruginosa Metabolite Database (PAMDB) to identify metabolites.

Lung Infection Model Results

Several AQ species were observed in the infected lung tissue after MALDI-MSI analysis, the most abundant being C_6H_{13} and C_7H_{15} PQS/AQNO. Both AQ species are more intense in bronchus regions of the lung, as this is the location of the infection[5]. Both AQs are more intense in HemO TM and QM models when compared to PAO1 models, perhaps suggesting a greater extent of infection. In addition, biliverdin was also observed in all lung infection models, indicating heme catabolism, which is essential for *Pseudomonas* propagation.



Future Directions

In the future, metabolomic differences between the four infection models will be further assessed using Metaspace, an online metabolite annotation platform for analyzing MSI data. In addition, DESI-MSI will also be used to further characterize these infection models. The methodology developed using the infection models will be translated to lung samples of RILI and could be used to probe the effect or RILI on infection and immune response in the lung in future studies.

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