Identification of Mycoplasma spp. using MALDI-TOF Mass Spectrometry

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Outline

- Background Information
- Introduction
- Methods
- Results
- Conclusion
- Next Steps
Mycoplasma spp.

- Lack a cell wall
- Among the smallest genomes
- Take 3-15 days to grow
- “Fried egg” colony morphology

From: www.venanet.pl
Bovine Mycoplasmosis

- Cause pneumonia, mastitis, and arthritis
- Mycoplasma bovis is the most pathogenic species
- M. bovirhinis, M. bovigenitalium, M. canadense, M. californicum are other relevant species
- Some species are normal flora in upper respiratory tract
Current Diagnostic Protocol

- Sample plated to Mycoplasma Agar and added to Mycoplasma Broth with inhibitor
- Broth plated to Mycoplasma Agar after 3-4 days of incubation, broth frozen
- If positive growth on direct plate or plate from broth, original broth sent to Molecular for M. bovis PCR
MALDI-TOF Mass Spectrometry

- Colony smeared on metal plate
- Matrix reagent crystallizes proteins
- Laser ionizes crystallized proteins and detector measures mass and time of flight (z)
- Profile of proteins built (m/z) and compared to library of organisms
- Score given based on similarity of profile of new isolate to profile of library isolate
Project Outline

- **Part 1:**
  - Confirm identity of standard isolates using multiple identification methods

- **Part 2:**
  - Collect all bovine Mycoplasma isolates from cases
  - Run isolates through MALDI-TOF Mass Spec
Identification of Standard Isolates

- 13 different standards used
- Originally identified by Dr. Ricardo Rosenbusch via Fluorescent Ab
- Sent for Molecular 16S and for PCR by Dr. Jessie Trujillo
- MALDI done on all standards
- Current library has only 18 Mycoplasmas, 7 of those significant to bovine cases
## Standards and Strain ID

<table>
<thead>
<tr>
<th>Standard</th>
<th>Strain</th>
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<tbody>
<tr>
<td><em>Acholeplasma laidlawii</em></td>
<td>B</td>
</tr>
<tr>
<td><em>Mycoplasma alkalescens</em></td>
<td>PG 31 p11C1</td>
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<tr>
<td><em>Mycoplasma arginini</em></td>
<td>G 230 (Ca 767a P3)</td>
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<tr>
<td><em>Mycoplasma bovigenitalium</em></td>
<td>PG 11</td>
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<tr>
<td><em>Mycoplasma bovirhinis</em></td>
<td>352i p13</td>
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<tr>
<td><em>Mycoplasma bovis</em></td>
<td>Jasper</td>
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<tr>
<td><em>Mycoplasma bovis</em></td>
<td>SP M 23 8/7 W23</td>
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<td><em>Mycoplasma bovis</em></td>
<td>M 23</td>
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<tr>
<td><em>Mycoplasma bovoculi</em></td>
<td>C 52</td>
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<td><em>Mycoplasma califomicum</em></td>
<td>Cs 687 P 6</td>
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<td><em>Mycoplasma canadense</em></td>
<td>275C p 4</td>
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<td><em>Mycoplasma canis</em></td>
<td>P 6C 2</td>
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<tr>
<td>Serogroup 7</td>
<td>PG 50 p 3</td>
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Results for Standards

- 8 of 13 identified correctly by all methods
- 4 others were identified the same by every method but the original (F Ab)
  - *M. arginini* as *M. bovis*, *M. bovirhinis* as *M. alkalescens*, *M. bovoculi* as *M. alkalescens*, *M. califomicum* as *M. bovis*
- Serogroup 7 is the Mycoides group which could be distinguished using these methods
Case Isolates

- 35 isolates used, 13 had NRID
- 13 nasal swabs, 12 lungs, 4 milks, 3 eyes, 2 joints, and 1 trachea
- All but the 4 milks were sent for PCR
- 15 positive for M. bovis by PCR
Case Isolate Statistics

- When MALDI identified as *M. bovis*, 12/12 (100%) confirmed by PCR
- When MALDI identified as other species, 7/9 (78%) confirmed *M. bovis* negative by PCR
- Misidentified as not *M. bovis* when compared to PCR results 2/15 (13%)
- One other isolate not sent for PCR, but identified as *M. bovis* (basic milk)
Conclusions

- Misidentification of standards due to similarity of organisms

- M. bovis can certainly be identified reliably through MALDI since it was 100% when identified as M. bovis

- Also identifies as not M. bovis fairly reliably, even with small MALDI library

- No apparent difference between smear and smear with formic acid
Next Steps

- Add many more Mycoplasma to the MALDI library, preferably from different sources (more milk isolates)
- Look more closely into smear vs formic acid and also full extraction
- Send non-M. bovis isolates for 16S (or similar) to confirm correct identification
Works Consulted

