Identification of *Avibacterium paragallinarum* by MALDI-TOF mass spectrometry

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Introduction

*Avibacterium paragallinarum* (AVP) is the causative agent of infectious coryza (IC), a severe disease of the upper respiratory tract in broilers, layers and breeders, resulting in serious economic losses. Reliable identification of AVP in routine diagnostics is complicated; advanced diagnostic techniques are required. In this study, we evaluated matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for identification of clinical AVP isolates by using the Bruker MALDI-TOF MS Biotyper system with the db7311 database (V7 database).

Materials and Methods

Test set:
- 12 AVP reference strains1
- 71 AVP and 15 non-AVP from outbreaks suspected of IC (commercial and backyard poultry)
  - Classification as AVP and non-AVP based on molecular identification (species-specific PCR2 and/or sequencing of 16S rRNA3)

Culture:
- 6% Sheep blood agar (SBA) inoculated with a nurse culture (*Staphylococcus lentus*)
- Chocolate agar (CHOC)
  - 37°C, CO2-enriched atmosphere

MALDI-TOF MS:
- MALDI Biotyper Microflex LT instrument (Bruker Daltonics, Bremen, Germany)
- MALDI Biotyper database db7311 (V7 database) (Bruker), containing representatives of the following five *Avibacterium* species: *A. avium*, *A. endocarditidis*, *A. gallinarum*, AVP, and *A. volantium*
- Colony material from both 24 h-old and 48 h-old cultures on SBA and CHOC
  - Tested in triplicate, using the direct transfer method, following the standard protocol of the manufacturer
  - In case of a no reliable identification, an on-target extraction was performed, in triplicate, by overlaying the spot with 70% formic acid according to the manufacturer’s recommendations
  - Results of triplicate testing were interpreted according to an in-house decision tree, with the following possible outcomes:
    - AVP
      - presumptive AVP/non-valid test result, confirm by PCR
    - non-AVP
      - non-AVP (perhaps) other *Avibacterium* species
      - continue incubation for another 24 h (only for 24 h-old cultures) and retest

Conclusion

Overall, the MALDI method tested proved to be highly reliable for identification of AVP with 48-h-old cultures, preferably on CHOC, and therefore, is an additional valuable tool for the identification of AVP next to species-specific PCR and 16S rRNA sequencing techniques.

References

1 Antimicrobial susceptibility of *Avibacterium paragallinarum* isolates from outbreaks of infectious coryza in Dutch commercial poultry flocks. embryol. 2018;227(107):137-143. doi: 10.1159/000486099

**Table 1** Results of identification of AVP (n=83) and non-AVP (n=15) by using the Bruker MALDI-TOF MS Biotyper system with the db7311 database and interpreting MALDI results by an in-house decision tree

<table>
<thead>
<tr>
<th>TIME OF INCUBATION</th>
<th>AGAR</th>
<th>CORRECT</th>
<th>INCORRECT</th>
<th>RETEST AFTER ANOTHER 24 h OF INCUBATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>SBA</td>
<td>75%</td>
<td>0%</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>CHOC</td>
<td>78%</td>
<td>1%</td>
<td>21%</td>
</tr>
<tr>
<td>48 h</td>
<td>SBA</td>
<td>98%</td>
<td>2%</td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td>CHOC</td>
<td>99%</td>
<td>1%</td>
<td>n.a.*</td>
</tr>
</tbody>
</table>

* a peacock isolate (NAD-independent) was identified by MALDI as AVP but this was not confirmed by 16S rRNA sequence analysis;
  - a chicken isolate (NAD-independent) was identified by MALDI in AVP but this was not confirmed by 16S rRNA sequence analysis.
  - not applicable

**Identification by MALDI-TOF MS**

**Chicken showing clinical signs of IC**

**Culture of NAD+ AVP on 6% SBA inoculated with a nurse culture**