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 strains[1]
- 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 PCR[2] and/or sequencing of 16S rRNA[3])

Culture:

- 6% Sheep blood agar (SBA) inoculated with a nurse culture (*Staphylococcus lentus*)
- Chocolate agar (CHOC)
  - 37°C, CO_2-enriched atmosphere

MALDI-TOF MS:

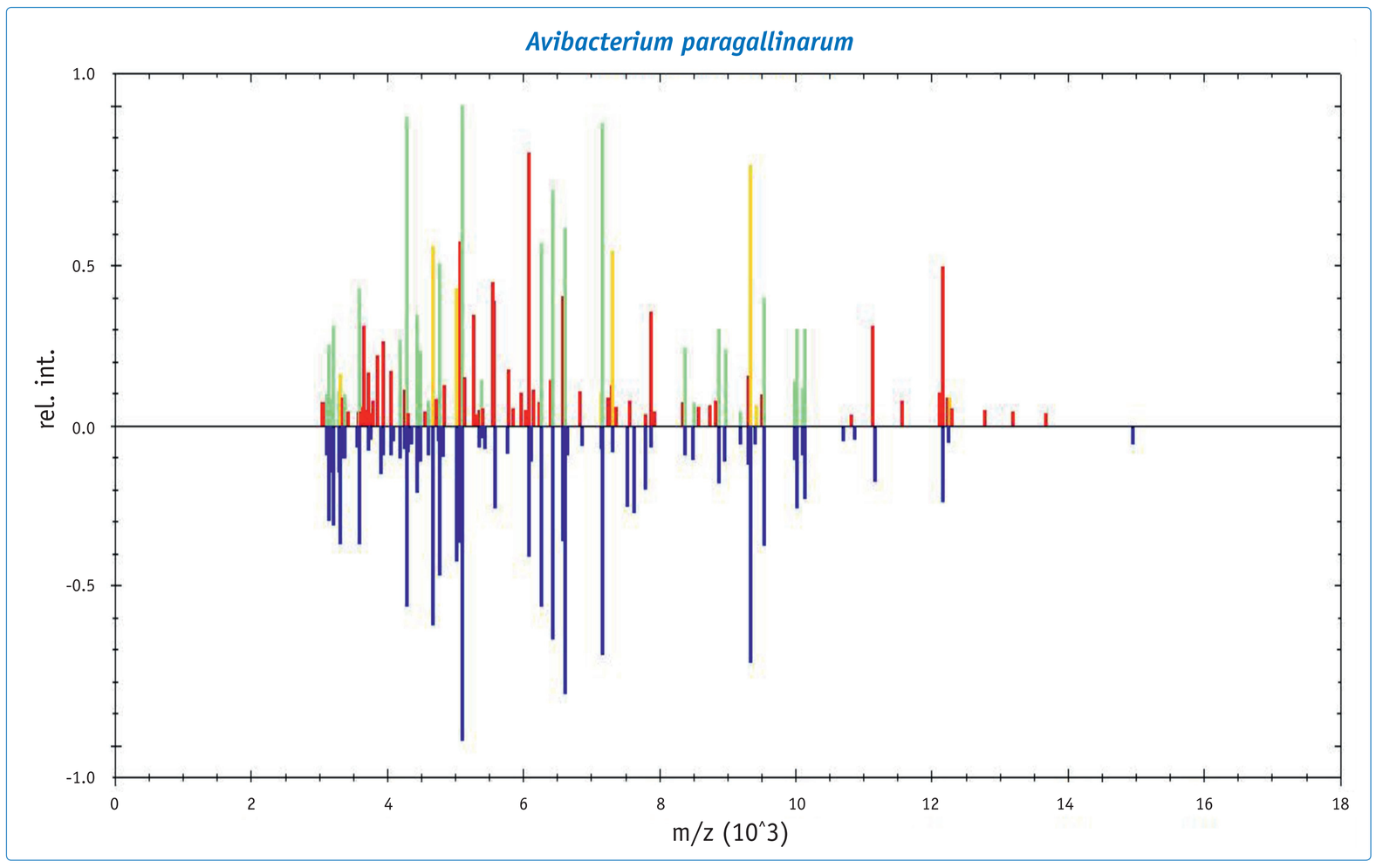
- MALDI Biotyper Microflex LT instrument (Bruker Daltonics, Bremen, Germany)
- MALDI Biotyper database db7311 (V7 database) (Bruker), containing representatives of the following five *Avibacterium* species: *Av. avium*, *Av. endocarditidis*, *Av. gallinarum*, AVP, and *Av. volantium*
  - Colony material 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

Results

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

Time of incubation	Agar	Correct (AVP as AVP; non-AVP as non-AVP)	Incorrect (AVP as non-AVP; non-AVP as AVP)	Retest after another 24 h of incubation
24 h	SBA	75%	0%	25%
	CHOC	78%	1%[a]	21%
48 h	SBA	98%	2%[a,b]	n.a.*
	CHOC	99%	1%[a]	n.a.

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



Conclusion

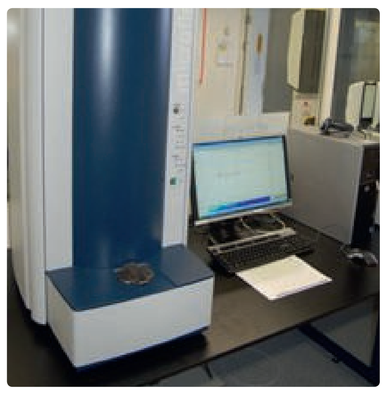
Overall, the MALDI method tested proved to be highly reliable for identification of AVP with 48-h old cultures, preferably on CHOC, and therefore, is an additional valuable tool for the identification of AVP next to species-specific PCR and 16S rRNA sequencing techniques.

References

[1] Antimicrobial susceptibility of *Avibacterium paragallinarum* isolates from outbreaks of infectious coryza in Dutch commercial poultry flocks, 2008-2017. Heuvelink A, Wiegel J, Kehrenberg C, Dijkman R, Soriano-Vargas E, Feberwee A. Vet Microbiol. 2018 Apr;217:135-143. doi: 10.1016/j.vetmic.2018.03.008

[2] Identification and characterization of Dutch *Avibacterium paragallinarum* isolates and the implications for diagnostics. Feberwee A, Dijkman R, Buter R, Soriano-Vargas E, Morales-Erasto V, Heuvelink A, Fabri T, Bouwstra R, de Wit S. Avian Pathol. 2019 Jul 24:1-8. doi: 10.1080/03079457.2019.1641178

[3] Phylogenetic positions of *Clostridium chauvoei* and *Clostridium septicum* based on 16S rRNA gene sequences. Kuhnert P., Capaul S.E., Nicolet J. and Frey J. Int. J. Syst. Bacteriol. 1996 Oct;46(4):1174-1176. doi: 10.1099/00207713-46-4-1174



Identification by MALDI-TOF MS

Chicken showing clinical signs of IC

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

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