Evaluation of a cultural approach to dissect putative mixed cultures of two *Brachyspira* species as suggested by conflicting PCR and MALDI-TOF-MS results

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Introduction

Pathogenic species of Brachyspira spp. affecting poultry can cause diarrhea, weight loss and can also reduce the capability of hens to lay eggs. Therefore these species of Brachyspira can reduce the efficiency of poultry farming. Differentiation of Brachyspira is difficult and a matter of controversy. A diagnostic gold standard is not available. Noteworthy, Brachyspira do not form single colonies on most solid media, such as Columbia blood agar, which makes it very difficult to prove the isolation of a mixed culture of two Brachyspira species.

Screening of reference strains within a recent doctoral thesis suggested that a novel *abg*B PCR is specific for *B. pilosicoli*. Furthermore, the tryptophanase gene tnaA was only detected in *B. intermedia* and *B. hyodysenteriae* reference strains. However, a few Brachyspira isolates from poultry generated conflicting PCR and MALDI-TOF-MS results. Specifically, the *abg*B or *tna*A gene was detected by PCR in isolates identified as *B. innocens* or *B. murdochii* by MALDI-TOF-MS.

The aim of this study was to investigate the hypothesis that these conflicting results are related to a putative mixed culture of two *Brachyspira* species, e. g. by *B. innocens* and *B. pilosicoli*.

PCR- and MALDI-TOF-MS based methods were conducted afterwards to determine the bacterial strain on each agar plate. In addition, the mode of growth of Brachyspira on dog blood agar plates was investigated.

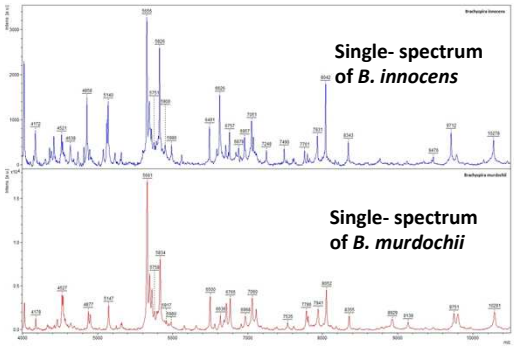
Experimental Approach

A Production of OXOID-anaerobe basal agar



To analyse the preexisting bacterial culture presumably consisting of different bacterial strains, single colonies were extracted from the culture and placed on anaerobe basal agar plates with 10 % horse blood from Sweden (OXOID) which improves the growth of single colonies and simplifies a differentiation of the culture.

B MALDI-TOF MS analysis



C MP-PCR differentiation of pathogenic brachyspira

1) *nox* – PCR
- nox gene
- 939 bp amplicon

➢ Brachyspira sp.

2) *tna*A – PCR
- Tryptophanase gene
- 325 bp amplicon

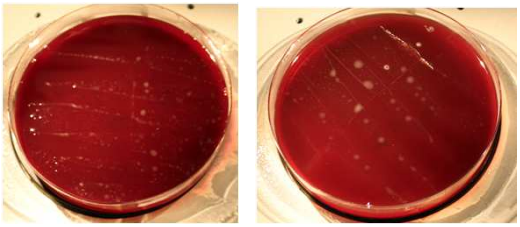
➢ B. intermedia
➢ B.hyodysenteriae

3) *abg*B - PCR
- hippurat- hydrolase gene
- 744 bp amplicon

➢ B. pilosicoli
➢ (B. alvinipulli)

Results

A Growth of single colonies on OXOID-anaerobe basal agar plates



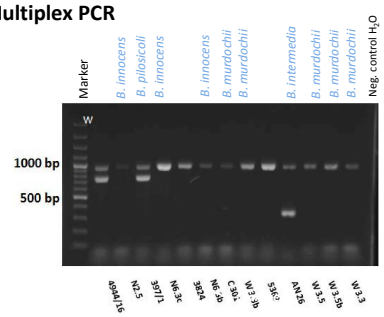
B. murdochii on horseblood and dogblood agar

Three different colonies (a,b,c) from each original putative mixed culture on anaerobe basal agar plates with 10 % horse or dog blood were subcultured. Finally, five generations of subcultures were produced.

B MALDI-TOF-MS and PCR-MS

Bacterial culture	Type of blood used for agar plate	MALDI-TOF-MS result	MALDI-TOF-MS score	MALDI-TOF-MS score Harms Bruker Database/ Extended Database	PCR-MS nox gene detected
W 3.5	dog & horse	B. murdochii	2,1	1,52 / 2,5	×
W 3.5a	horse	discarded			
W 3.5b	horse	B. murdochii	2,32		×
W 3.5c	horse	contaminated			
W 3.3	dog			1,61 / 2,49	×
W 3.3a	horse	B. murdochii	2,05		
W 3.3b	horse	B. murdochii	2,33		×
W 3.3c	horse	B. murdochii	2,18		
N 6.3	dog	B. innocens	2,12	1,86 / 2,75	
N 6.3a	horse	B. innocens	2,12		
N 6.3b	horse				×
N 6.3c	horse	B. innocens	2,05		×

C Multiplex PCR



Nox-gene: NADH oxidase
abgB-gene: hippurathydrolase
tnaA-gene: Tryptophanase

Bacterial culture	nox-gene	abgB	tnaA
N 2.5*	x		
397/1*	x	x	
N 6.3 c[2]	x		
N 6.3 b[2]	x		
C 301*	x		
W 3.3 b[1]	x		
AN 26/93*	x		x
W 3.5[1]	x		
W 3.5 b[1]	x		
W 3.3[1]	x		

[1] tnaA gene diagnosed by M. Harms

[2] abgB gene diagnosed by M. Harms

* control strains

Conclusions

Culturing of *Brachyspira* isolates on anaerobe basal agar plates with 10% horse or dog blood generated single colonies which made subculturing and further differentiation of single colonies possible. All subclones were nox+, abgB- and tnaA- in the MP-PCR and were diagnosed as *B. Innocens* or *B. murdochii* in MALDI-TOF MS analysis.

As the original isolates showed conflicting results we postulate generation of pure cultures of *B. innocens* or *B. murdochii* through culturing on anaerobe basal agar with horse or dog blood. However, we did not demonstrate that the original isolates contained also *B. pilosicoli* or *B. intermedia*.

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