The present study was designed to characterize phenotypically and genotypically nine *Arcanobacterium abortisuis* strains collected from specimen of pigs in a period of nine years. All nine *A. abortisuis* strains and *A. abortisuis* reference strain DSM 19515 displayed a synergistic hemolytic reaction with *Staphylococcus aureus* $\beta$-hemolysin, *Rhodococcus equi*, and *Arcanobacterium haemolyticum* indicator strains and showed the typical biochemical properties of this species. The species identity could be confirmed by identification and sequencing of the 16S–23S rDNA intergenic spacer region (ISR), which appeared to be a useful target for genotypic characterization of this bacterial species. The *A. abortisuis* strains of the present study were isolated from specimen of pigs together with various other bacterial species indicating that the pathogenic importance of this newly described species remains to be elucidated.

**Keywords:**

*Arcanobacterium abortisuis*, Pig, Phenotypic properties, 16S–23S rDNA intergenic spacer region

1. Introduction
At present genus *Arcanobacterium* (A) consist of nine species namely *A. haemolyticum* and *A. bernardiae* which generally cause infections in humans (Collins et al., 1982; Funke et al., 1995) and *A. pyogenes, A. phocaen, A. bonasi, A. bialowiezense, A. hippocoleae, A. pluranimalium* and *A. abortisuis* which could mainly be recovered from infections of various animals (Lämmler and Hartwigk, 1995; Ramos et al., 1997; Lawson et al., 2001; Hoyles et al., 2002; Lehnen et al., 2006; Azuma et al., 2009). In 2009, Azuma et al., characterized the novel species *A. abortisuis*. The species description was based on a single strain isolated from a placenta of a sow following an abortion. According to a proposal of Yassin et al. (in press) *A. abortisuis* should be reclassified in the newly described genus *Trueperella* as *T. abortisuis*.

The aim of the present study was to further characterize this novel species *A. abortisuis* by investigating nine *A. abortisuis* strains isolated from specimen of nine pigs over a period of nine years during routine microbiological diagnostic by phenotypic properties and by sequencing the 16S–23S rDNA intergenic spacer region (ISR).

2. Materials and methods

2.1. Bacterial cultures and phenotypic properties

A total of 10 bacterial cultures were used in this study.

The cultures included the reference strain *A. abortisuis* DSM 19515 and nine additional strains described in the present study. Further data about the origin of the nine strains are summarized in Table 1. The nine strains were investigated for cultural and biochemical properties and for CAMP-like activities as described (Hassan et al., 2009; Ülbegi-Mohyla et al., 2009, 2010).
2.2. Genotypic properties

For molecular identification the bacterial strains of the present study were investigated by amplification and sequencing of the ISR as described recently (Hassan et al., 2008, 2009).

3. Results and discussion

All nine bacterial cultures investigated in the present study could be identified phenotypically and genotypically as *A. abortisuis*. The *A. abortisuis* strains, also including reference strain *A. abortisuis* DSM19515, could be cultivated under aerobic conditions, microaerobic conditions in a candle jar and under anaerobic conditions and produced a narrow zone of complete hemolysis on sheep and rabbit blood agar. Cultivation of the bacteria under anaerobic conditions and by microaerobic conditions in a candle jar, compared to aerobic conditions, resulted in a slightly enhanced growth of the bacteria. However, according to the information given by Azuma et al. (2009) *A. abortisuis* is strictly anaerobic. The degree of hemolysis of the *A. abortisuis* strains and the reference strain *A. abortisuis* DSM 19515 on sheep blood agar appeared to be slightly enhanced after cultivation under anaerobic conditions.

By determination of synergistic CAMP-like activities *A. abortisuis* displayed synergistic hemolytic reactions in close proximity of β-hemolytic *Staphylococcus aureus*, *Rhodococcus equi* and *A. haemolyticum* indicator strains.
The synergistic and antagonistic hemolytic reactions of the remaining eight species of genus Arcanobacterium had been described in detail (Ülbegi-Mohyla et al., 2009). In addition A. abortisuis did not cause liquefaction of Löffler agar and showed no cross reaction with group G Streptococcus antisera. Both properties are well known for A. pyogenes (Bisping and Amtsberg, 1988; Lämmler and Hartwigk, 1995). The biochemical properties of the A. abortisuis strains of the present study, determined with the Api–Coryne test system, with tablets containing various substrates and with methylumbelliferyl conjugated substrates generally corresponded to the findings of Azuma et al. (2009) and are summarized in Table 2. In addition all A. abortisuis strains investigated appeared to be positive for DNase and negative for catalase, hyaluronidase and acetoin (Table 2). The result for catalase also confirmed the findings of Azuma et al. (2009).
The nine *A. abortisuis* strains and *A. abortisuis* DSM 19515 could be identified genotypically by amplification of ISR and part of the 16S rDNA and 23S rDNA yielding for all 10 *A. abortisuis* strains an identical amplicon size of approximately 580 bp. According to Hassan et al. (2008) a comparable ISR-PCR of the other eight species of genus Arcanobacterium displayed amplicons with approximate sizes of 580–620 bp and an ISR sequence length between 333 and 380 bp. Sequencing ISR of the *A. abortisuis* strains of the present study revealed an ISR sequence length between 333 and 335 bp. A
dendrogram analysis of the ISR sequences of the nine *A. abortisuis* strains, *A. abortisuis* DSM 19515 and the remaining eight species of genus Arcanobacterium is shown in Fig. 1.

All nine *A. abortisuis* strains, isolated from the urogenital tract of pigs with varying clinical symptoms, were recovered together with various other bacteria indicating that the pathogenic importance of *A. abortisuis* remains questionable. None of the strains was isolated from an abort which was originally described by Azuma et al. (2009). However, the phenotypic and genotypic properties described in the present study might help to improve a future diagnostic of *A. abortisuis* and might elucidate the role this species plays in infections of pigs, other animals and possibly in humans.

References


