Identification of *Trueperella (Arcanobacterium) bernardiae* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis and by species-specific PCR

*Trueperella (Arcanobacterium) bernardiae* was first described as a probable opportunistic pathogen in 1987. It was among isolates from a variety of clinical specimens in the Centers for Disease Control and Prevention described as group 2 coryneform bacteria (Na’Was et al., 1987). It was finally classified in the genus *Arcanobacterium* by Ramos et al. (1997). However, Yassin et al. (2011) proposed that *Arcanobacterium bernardiae* togeth with *Arcanobacterium abortisuis*, *Arcanobacterium pyogenes*, *Arcanobacterium bialowiezense* and *Arcanobacterium bonasi* should be reclassified into the newly described genus *Trueperella*. *T. bernardiae* has been reported as causative agent of urinary tract infections (Leven et al., 1996; Lepargneur et al., 1998), from a patient suffering from septic arthritis (Adderson et al., 1998), from necrotizing fasciitis (Clarke et al., 2010), from coinfection with *Staphylococcus aureus* from chronic osteitis (Bemer et al., 2009) and more recently from bacteraemia of a patient with a deep soft tissue infection (Weitzel et al., 2011). However, the latter could be identified by partial sequencing of the 16S rRNA gene but not by classical biochemical tests, indicating that additional species characteristics would assist a future identification of this bacterial species.

The present study was designed to identify this previously described *T. bernardiae* strain H101 isolated from bacteraemia and type strain *T. bernardiae* DSM 9152 by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis and by sequencing of the gene encoding the β-subunit of RNA polymerase and the 16S–23S rRNA intergenic spacer region (ISR). MALDI-TOF MS analyses were performed according to the extraction protocol of Bruker Daltonik. A few colonies of freshly cultured bacteria were suspended into 75% ethanol. After centrifugation, the pellet was resuspended in 30 μl 70% formic acid and with the same volume of pure acetonitrile. The suspension was centrifuged and 1 μl of the supernatant was transferred to a polished steel MALDI target plate (Bruker Daltonik) and allowed to dry at room temperature. The sample was overlaid with 1 μl matrix (10 mg a-cyano-4-hydroxy-cinnamic acid ml⁻¹ in 50% acetonitrile/2.5% trifluoroacetic acid). Mass spectra were acquired using a microflex mass spectrometer (Bruker Daltonik) in the linear mode and a mass range of 2–20 kDa using the automated functionality of flexControl 3.0 software (Bruker Daltonik). At least 20 raw spectra were used to generate a main spectrum. The main spectrum of the *T. bernardiae* strain H101 analysed in the present study was matched to the database included in the MALDI Biotyper 2.0 software package (version 3.1.1.0, 3740 entries) and to the newly acquired main spectra of 11 reference strains representing nine species of the genera *Arcanobacterium* and *Trueperella* (Hijazin et al., 2011). The software calculates a similarity score [log (score)] by calculation of a value considering the proportion of matching peaks between the unknown spectrum and the main spectrum of the database, the frequency of peaks in multiple measurements as well as the consistency of the peak intensities between these spectra. The logarithmized score values range from 0 (no similarity) to 3 (absolute identity). Log (score) values ≥2 are rated as identification of bacteria at the species level. Log (score) values ≥1.7 and <2.0 are considered as identification of microorganisms at least on the genus level. Log score values <1.7 indicate that a spectrum is not suitable for identification by the MALDI Biotyper. Sequencing of the molecular targets rpoB and the ISR was performed as described previously (Hassan et al., 2008; Ülbegi-Mohyla et al., 2010). Both *T. bernardiae* and type strains representing eight species of the genera *Arcanobacterium* and *Trueperella* (Ülbegi-Mohyla et al., 2010) were additionally investigated by a newly designed PCR-mediated amplification of a *T. bernardiae*-specific region of the 16S rRNA gene and the ISR. The species-specific region could be amplified with an expected size of 160 bp by using the oligonucleotide primers Abe-16S rDNA-F (5’-CTC TCT TCT AAG GAG CCT CAT-3’) and Abe-ISR-R (5’-CAA ACA AAC AGC CTG AAA ACT TG-3’). The PCR program was carried out as follows: one step of 10 min at 95 °C (initial denaturation), 30 cycles with one cycle consisting of 30 s at 95 °C (denaturation), 40 s at 57 °C (annealing) and 45 s at 72 °C (elongation) and one step of 7 min at 72 °C (final elongation), using the GeneAmp PCR system 2400 (Perkin-Elmer). The oligonucleotide primers used in the present study were synthesized by Eurofins MWG/Operon.

As shown by numerous authors, MALDI-TOF MS appears to be a powerful tool for species classification of a broad spectrum of Gram-positive and Gram-negative bacteria (Seng et al., 2009; Murray, 2010; Bizzini et al., 2011). Comparable to the previously conducted MALDI-TOF MS analysis of *Trueperella (Arcanobacterium) abortisuis* (Hijazin et al., 2011), MALDI-TOF MS allowed the identification of *T. bernardiae* strain H101 of the present study to the species level, matching to *T. bernardiae* type strain DSM 9152 with a log score value of 2.37. A typical dendrogram of the main spectra of *T. bernardiae* H101 of the present study and nine type strains representing nine species of the genera *Arcanobacterium* and *Trueperella* is shown in Fig. 1.
T. bernardiae H101 of the present study was additionally identified genotypically by amplification and sequencing of the \textit{rpoB} gene (partial sequence) and the ISR (GenBank accession nos FR873576 and FR873575, respectively). Both sequences were 100\% identical to \textit{rpoB} and ISR sequences of \textit{T. bernardiae} DSM 9152\textsuperscript{T} (GenBank accession nos FN550366 and EU194562, respectively) (data not shown).

As previously described for \textit{Trueperella bialowiezensis} (\textit{Arcanobacterium bialowiezense}), \textit{Trueperella (Arcanobacterium) pyogenes} and \textit{Trueperella (Arcanobacterium) abortisuis} (Hassan et al., 2008, Hijazin et al., 2010, 2011; Ülбеги-Мохила et al., 2010), sequencing the bacterial ISR also allowed the design of \textit{T. bernardiae} 16S rRNA gene and ISR specific oligonucleotide primers which could be used for PCR-mediated identification of \textit{T. bernardiae} H101 of the present study and \textit{T. bernardiae} DSM 9152\textsuperscript{T}. The \textit{T. bernardiae} specific PCR yielded no cross-reaction with the control strains representing eight other species of the genera \textit{Arcanobacterium} and \textit{Trueperella} (Fig. 2), indicating the usefulness of the species-specific PCR for identification of \textit{T. bernardiae}. The MALDI-TOF MS analysis and the genotypic results of the present study might help to improve future identification of \textit{T. bernardiae} and might help to elucidate the role that this rarely isolated species plays in infections of humans and possibly of animals.

**Fig. 1.** A score oriented dendrogram of MALDI-TOF main spectra profiles was generated using the MALDI Biotyper 3.0 software with the following settings for peak picking: lower bound 3000, upper bound 15 000, maximum peaks 100 and threshold 0.001.

**Fig. 2.** Typical species-specific amplicons of 16S–ISR rDNA of \textit{T. bernardiae} H101 (lane 1) and \textit{T. bernardiae} DSM 9152\textsuperscript{T} (lane 2) with an approximate size of 160 bp. Lanes 3 and 4, negative reaction of \textit{T. (A) pyogenes} DSM 20630\textsuperscript{T} and \textit{T. (A) abortisuis} DSM 19515\textsuperscript{T}, respectively. Lane M, GeneRuler 100 bp DNA ladder (Fermentas).

Correspondence: C. Lämmler
(Christoph.Laemmler@vetmed.uni-giessen.de)


