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Short Communication

Identification of *Arcanobacterium pyogenes* isolated by post mortem examinations of a bearded dragon and a gecko by phenotypic and genotypic properties

H. Ülbegi-Mohyla[1], M. Hijazin[1], J. Alber[1], C. Lämmler[1,*], A. A. Hassan[2], A. Abdulmawjood[3], E. Prenger-Berninghoff[4], R. Weiß[4], M. Zschöck[5]

[1]*Institut für Pharmakologie und Toxikologie, Justus-Liebig-Universität Gießen, Frankfurter Str. 107, 35392 Gießen, Germany*
[2]*GD Animal Health Service, Department of Bacteriology and Parasitology, Postbus 9, 7400 AA Deventer, The Netherlands*
[3]*Institut für Tierärztliche Nahrungsmittelkunde, Justus-Liebig-Universität Gießen, Frankfurter Str. 92, 35392 Gießen, Germany*
[4]*Institut für Hygiene und Infektionskrankheiten der Tiere, Justus-Liebig-Universität Gießen, Frankfurter Str. 85-91, 35392 Gießen, Germany*
[5]*Landesbetrieb Hessisches Landeslabor, Schubertstr. 60, 35392 Gießen, Germany*

The present study was designed to identify phenotypically and genotypically two *Arcanobacterium* (*A.*) *pyogenes* strains isolated by post mortem examinations of a bearded dragon and a gecko. The *A. pyogenes* strains showed the typical biochemical properties and displayed CAMP-like synergistic hemolytic activities with various indicator strains. The species identity could be confirmed genotypically by amplification and sequencing of the 16S rDNA gene and, as novel target gene, by sequencing of the beta subunit of RNA polymerase encoding gene *rpo*B, of both strains and of reference strains representing nine species of the genus *Arcanobacterium*. The species identity of the two *A. pyogenes* strains could additionally be confirmed by PCR mediated amplification of species specific parts of the 16S-23S rDNA intergenic spacer region, the pyolysin encoding gene *plo* and by amplification of the collagen-binding protein encoding gene *cbpA*. All these molecular targets might help to improve the future identification and further characterization of *A. pyogenes* which, as demonstrated in the present study, could also be isolated from reptile specimens.

Keywords: *Arcanobacterium pyogenes*, bearded dragon, gecko, *rpo*B, 16S rDNA

Arcanobacterium (*A.*) *pyogenes* is a well-known pathogen of domestic ruminants and pigs causing mastitis, abortion and a variety of pyogenic infections [10]. As summarized by Jost and Billington [7] this bacterial pathogen is also able to cause disease in a large number of various animal species. However, at present little is known about *A. pyogenes* isolated from reptiles.

A total of 13 bacterial cultures were used in this study. The cultures included 11 reference strains of nine species of the genus *Arcanobacterium* [5,6,12], and the strains *A. pyogenes* 734 and *A. pyogenes* 4984 described in the present study. *A. pyogenes* 734 was isolated from the lung of a bearded dragon (*Pogona vitticeps*) together with γ-hemolytic streptococci, *Pseudomonas* spp. and coliform bacteria; *A. pyogenes* 4984 was isolated from the intestine of a gecko together with α and β-hemolytic streptococci, *Acinetobacter* spp. and yeasts. Both animals were investigated by post mortem examinations. *A. pyogenes* was isolated in large numbers from the lung and kidney of the bearded dragon and from the intestine, lung, liver and kidney of the gecko. The gecko showed signs of enteritis. The post mortem isolation of *A. pyogenes* in relatively large numbers from various organs led to a diagnosis of septicemia for both animals with *A. pyogenes* deemed the major causative agent. The *A. pyogenes* strains were investigated for cultural and biochemical properties using the Api-Coryne test system (bioMérieux, Germany), tablets containing various substrates (Inverness Medical, Germany), and 4-methylumbelliferyl-conjugated substrates (Sigma, Germany). The strains were also investigated for CAMP-like synergistic hemolytic activities, as previously described [6,12].

For molecular identification both *A. pyogenes* strains were investigated by 16S rDNA amplification and sequencing [6]. In addition, the beta subunit of RNA polymerase encoding gene *rpo*B of nine species of genus *Arcanobacterium* and both *A. pyogenes* strains were amplified using the oligonucleotide primers described by

*Corresponding author
Tel: +49-6419938406, Fax: +49-6419938409
E-mail: Christoph.Laemmler@vetmed.uni-giessen.de

Table 1. Oligonucleotide primer sequences and PCR conditions

Oligonucleotide primers	Sequence	Program*	Size of PCR product (bp)	References
1. 16S rDNA UNI-L	5′-AGAGTTTGATCATGGCTCAG-3′	1	1,403	[9]
2. 16S rDNA UNI-R	5′-GTGTGACGGGCGGTGTGTAC-3′			
3. *rpo*B-C2700F	5′-CGWATGAACATYGGBCAGGT-3′	2†, 3‡	446	[8]
4. *rpo*B-C3130R	5′-TCCATYTCRCCRAARCGCTG-3′			
5. 16S-23S-ISR§ (*A. pyogenes*)	5′-GTTTTGCTTGTGATCGTGGTGGTTATGA-3′	4	122	Present study
6. 16S-23S-ISR (*A. pyogenes*)	5′-AAGCAGGCCCACGCGCAGG-3′			
7. *plo*-Pyolysin	5′-CGATCCCTCTGGTGTACTTGC-3′	5	704	Present study
8. *plo*-Pyolysin	5′-GCTTGACAAAAATCTGGCGTCC-3′			
9. *plo*-Pyolysin	5′-GGCCCGAATGTCACCGC-3′	6	270	[3]
10. *plo*-Pyolysin	5′-AACTCCGCCTCTAGCGC-3′			
11. *cbpA*-collagen-binding protein	5′-CTTGAAATCGAACTTAAGGCTGG-3′	7	328	Present study
12. *cbpA*-collagen-binding protein	5′-ATCGCCAGTCACCTTAGACG-3′			

*PCR Program 1: ×1 (95°C, 600 sec), ×30 (95°C, 30 sec, 58°C, 60 sec, 72°C, 60 sec), ×1 (72°C, 420 sec). 2: ×1 (95°C, 600 sec), ×35 (94°C, 30 sec, 37°C, 30 sec, 72°C, 120 sec), ×1 (72°C, 600 sec). 3: ×1 (95°C, 600 sec), ×35 (94°C, 30 sec, 50°C, 30 sec, 72°C, 120 sec), ×1 (72°C, 600 sec). 4: ×1 (95°C, 600 sec), ×30 (95°C, 30 sec, 64°C, 15 sec, 72°C, 30 sec), ×1 (72°C, 420 sec). 5: ×1 (95°C, 600 sec), ×30 (95°C, 60 sec, 62°C, 60 sec, 72°C, 60 sec), ×1 (72°C, 420 sec). 6: ×1 (95°C, 600 sec), ×35 (94°C, 60 sec, 55°C, 60 sec, 72°C, 60 sec), ×1 (72°C, 300 sec). 7: ×1 (94°C, 600 sec), ×30 (95°C, 30 sec, 58°C, 30 sec, 72°C, 60 sec), ×1 (72°C, 420 sec). †For *Arcanobacterium* (*A.*) *bialowiezense* DSM 17162, *A. bonasi* DSM 17163, *A. haemolyticum* DSM 20595, *A. hippocoleae* DSM 15539, *A. phocae* DSM 10002, *A. phocae* DSM 10003. ‡For *A. abortisuis* DSM 19515, *A. bernardiae* DSM 9152, *A. pyogenes* DSM 20630, *A. pyogenes* DSM 20594 and both *A. pyogenes* strains of the present study. §16S-23S rDNA intergenic spacer region.

Khamis *et al*. [8] (Table 1). DNA extraction, sequencing and the alignment studies were performed as described previously [5,6]. In addition, both *A. pyogenes* strains were investigated by PCR-mediated amplification of a species specific region of the 16S-23S rDNA intergenic spacer region (ISR), by amplification of the pyolysin encoding gene *plo* described in the present study and by Ertas *et al*. [3], and by amplification of the collagen-binding protein encoding gene *cbpA*. The oligonucleotide primers used in this study were synthesized by MWG Biotech (Germany) and Operon (Germany). The oligonucleotide primer sequences, the thermal cycler PCR programs and the expected amplicon sizes are summarized in Table 1.

Both bacterial strains investigated in the present study were identifiable phenotypically and genotypically as *A. pyogenes*. In similarity to the *A. pyogenes* reference strains and to previous findings [12] both *A. pyogenes* strains displayed hemolytic properties on sheep and rabbit blood agar, a synergistic CAMP-like hemolysis with staphylococcal β-hemolysin, *Rhodococcus equi* and *Arcanobacterium hemolyticum* indicator strains and, described for the first time in the present study, a synergistic CAMP-like hemolysis with *A. phocae*.

Both *A. pyogenes* strains of the present study were additionally identifiable genotypically by amplification and sequencing of the 16S rRNA gene (GenBank accession number, FN394982 and FN394983) and by amplification and sequencing of the *rpo*B gene of both strains (FN550376, FN550377) which show an almost complete identity to the *rpo*B sequences of *A. pyogenes* DSM 20630 (FN550375) and *A. pyogenes* DSM 20594 (FN550374). The *A. pyogenes rpo*B sequences differed clearly from the *rpo*B sequences of reference strains of eight additionally sequenced species of the genus *Arcanobacterium* (FN550365-FN550373). Dendrogram analysis of the 16S rDNA and *rpo*B sequencing results are shown in Fig. 1. 16S rDNA and ISR sequencing had already been used to characterize *A. haemolyticum* isolates from horses [6] and ISR sequencing had been used to design ISR specific oligonucleotide primers for PCR mediated identification of *A. bialowiezense* and *A. bonasi* [5]. Comparable to 16S rDNA and ISR, *rpo*B gene sequencing of bacteria of the genus *Arcanobacterium* might represent a novel target for molecular characterization of bacteria of this genus.

In addition both strains reacted positively with the *A. pyogenes* ISR-specific oligonucleotide primers described in the present study (data not shown). *A. pyogenes* expresses a cholesterol-dependent cytolysin, designated as pyolysin, that is a well known major virulence factor of this species [1]. As a constant characteristic feature of *A. pyogenes*, the pyolysin encoding gene *plo* could be used for molecular identification of this species [2,3].

The *A. pyogenes plo* specific oligonucleotide primers described in the present study and by Ertaş *et al*. [3] could be used to amplify a corresponding gene of both *A. pyogenes* strains of the present study (data not shown)

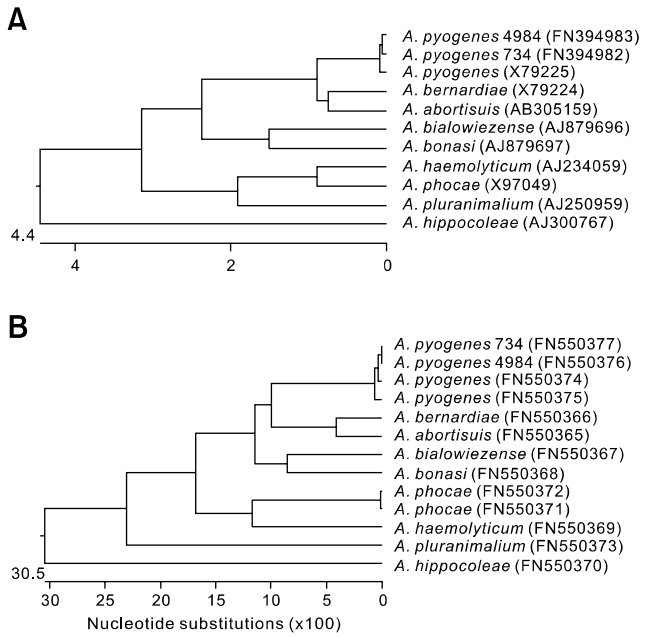

Fig. 1. Dendrogram analysis of 16S rRNA gene sequences (A) and *rpo*B gene sequences (B) of the *A. pyogenes* strains of the present study and of various *Arcanobacterium* spp. obtained from the NCBI GenBank and of sequencing results of the present study.

indicating that all three species specific oligonucleotide primer sets could successfully be used for molecular identification of this bacterial species.

Summarizing the potential virulence factors of *A. pyogenes*, Jost and Billington [7] described a collagen-binding protein cbpA which seems to promote the adhesion of these bacteria to collagen-rich tissues. As shown in the present study the virulence factor cbpA encoding gene *cbpA* seems to also be present in both *A. pyogenes* strains isolated from reptiles and in reference strain *A. pyogenes* DSM 20630 (ATCC 19411) of porcine origin. According to Esmay *et al*. [4] *cbpA* was found in *A. pyogenes* of bovine (49%), porcine (40%) and avian (100%) origin. Isolates from canines and felines did not carry *cbpA*. Silva *et al*. [11] were able to detect *cpbA* in all 57 investigated *A. pyogenes* strains of bovine origin.

To our knowledge the present study is the first detailed phenotypic and genotypic characterization of *A. pyogenes* strains isolated from specimens of a bearded dragon and a gecko. However, the usefulness of the *rpo*B gene as a target gene for identification of bacteria of the genus *Arcanobacterium* and the usefulness of the various species specific oligonucleotide primers for molecular identification and further characterization of *A. pyogenes* need to be investigated with a larger number of *A. pyogenes* strains of various origins.

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