Phenotypic and genotypic characteristics of *Trueperella* (*Arcanobacterium*) *pyogenes* isolated from lung abscesses of one-humped camels (*Camelus dromedarius*) in Jordan

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Abstract

Two strains of *Trueperella* (*Arcanobacterium*) *pyogenes* previously isolated from lung abscesses of two camels (*Camelus dromedarius*) in Jordan were identified phenotypically, by MALDI-TOF MS analysis and genotypically using *T.* (*A.*) *pyogenes* 16S-23S rDNA intergenic spacer region (ISR) and *T.* (*A.*) *pyogenes* superoxide dismutase A encoding gene *sod*A specific oligonucleotide primers. Both isolates could additionally be characterized by PCR-mediated amplification of several known and putative virulence factor encoding genes which revealed the presence of the genes *plo*, *nan*P and *fim*E but not *nan*H, *cbp*A and *fim*C for both isolates and the presence of *fim*A for one isolate. These results are the first report about genotypic properties of *T.* (*A.*) *pyogenes* isolated from camels.

Key words: *Arcanobacterium*, *Trueperella pyogenes*, one-humped camel, lung abscess.

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Introduction

According to a proposal of Yassin et al. (2011) *Arcanobacterium pyogenes* together with *Arcanobacterium abortisuis, Arcanobacterium bernardiae*, *Arcanobacterium bonasi* and *Arcanobacterium bialowiezense* should be reclassified in the newly described genus *Trueperella* and genus *Arcanobacterium* should be restricted to *Arcanobacterium haemolyticum*, *Arcanobacterium phocae* and *Arcanobacterium pluranimalium* and to *Arcanobacterium hippocoleae,* a phylogenetic neighbor of this group.

T. (*A.*) *pyogenes* is a worldwide pathogen of domestic ruminants and pigs, causing mastitis, abortion and a variety of pyogenic infections (Lämmler and Hartwigk 1995; Moore et al., 2010). As

summarized by Jost and Billington (2005), it is able to cause diseases in various other animal species, including camels. In one-humped camels *T.* (*A.*) *pyogenes* was isolated together with various other bacterial species from lung abscesses and from arthritic joints, both mostly from juvenile camels (Al-Tarazi, 2001; Bani Ismail et al., 2007).

However, at present little is known of the phenotypic and genotypic properties of *T.* (*A.*) *pyogenes* isolated from camels.

Materials and Methods

In the present study two previously isolated *T.* (*A.*) *pyogenes* obtained from lung abscesses of two camels (Al-Tarazi, 2001) were investigated phenotypically, by MALDI-TOF MS analysis and for various genotypic properties.

Determination of phenotypic properties, MALDI-TOF MS analysis and PCR-mediated identification and further characterization of the two *T.* (*A.*) *pyogenes* and type strains of genera *Trueperella* and *Arcanobacterium* was performed as described previously (Hijazin et al., 2011a, b, 2012).

Results and Discussion

Both *T.* (*A.*) *pyogenes* investigated in the present study were identified by determination of haemolysis and CAMP-like haemolytic reactions, by using the API Coryne test system and various other previously described tests (see Table 1), by MALDI-TOF MS analysis matching with log (score) values ≥ 2.0 and genotypically using *T.* (*A.*) *pyogenes* ISR and *T.* (*A.*) *pyogenes* gene *sod*A as molecular targets. These results corresponded to previously described properties of *T.* (*A.*) *pyogenes* of various origins (Ülbegi-Mohyla et al., 2010; Hijazin et al., 2011a).

Comparable to the present results MALDI-TOF MS had already been shown to be a rapid and reliable technique for identification of bacteria of genera *Arcanobacterium* and *Trueperella* (formerly known as genus *Arcanobacterium*) (Hijazin et al., 2011b, 2012) and could also be used for identification of a broad spectrum of bacterial species, including Gram positive and Gram negative cocci and rods, at the species and subspecies level (Murray, 2010).

Amplification of the known and putative virulence factor encoding genes revealed that both *T.* (*A.*) *pyogenes* of the present study carried the gene *plo* encoding pyolysin, gene *nanP* encoding neuraminidase NanP and gene *fim*E encoding fimbrial subunit FimE. The genes *nan*H, *cbp*A and *fim*C encoding neuraminidase NanH, collagen-binding protein CbpA and fimbrial subunit FimC, respectively, were not in either isolates. One *T.* (*A.*) *pyogenes* isolate expressed the fimbrial gene *fimA*. The phenotypic and genotypic properties of both isolates are summarized in Table 1.

Table 1. Physiological properties and putative virulence factor encoding genes of two *T.* (*A.*) *pyogenes* isolated from camels and two *T.* (*A.*) *pyogenes* reference strains

Biochemical properties	***T.* (*A.*) *pyogenes* (n = 2)**	***T.* (*A.*) *pyogenes* DSM 20630**	***T.* (*A.*) *pyogenes* DSM 20594**
Hemolysis	+(2)	+	+
CAMP-like hemolytic reactions	+(2)*	+	+
Nitrate reduction	-[1]	-[1]	-[1]
Pyrazinamidase	-[1]	-[1]	-[1]
Pyrrolidonyl arylamidase	(+)(2)[1]; +(2)[2]	+[1,2]	+[1, -2]
Alkaline phosphatase	(+)(2)[1]; +(2)[2]	-[1,2]	-[1,2]
β-Glucuronidase	+(2)[1, 3]	+[1,3]	+[1,3]
β-Galactosidase	+(2)[1, 3]	+[1,3]	+[1,3]
α-Glucosidase	+(2)[1, 3]	+[1,3]	+[1,3]
N-Acetyl-β-Glucosaminidase	+(2)[1, 3]	+[1,3]	+[1,3]
Esculin (β-Glucosidase)	-[1]	-[1]	-[1]
Urease	-[1]	-[1]	-[1]
Gelatine	+(2)[1]	+[1]	+[1]
Fermentation of:			
Glucose	+(2)[1]	+[1]	+[1]
Ribose	+(2)[1]	+[1]	+[1]
Xylose	+(2)[1]	+[1]	+[1]
Mannitol	-[1]	-[1]	-[1]
Maltose	+(2)[1]	+[1]	+[1]
Lactose	+(2)[1]	+[1]	+[1]
Saccharose	+(2)[1]	+[1]	+[1]
Glycogen	-[1]	+[1]	-[1]
α-Mannosidase	-[2]	-[2]	-[2]
Catalase	-	-	-
Caseinase	+(2)	+	+
Starch hydrolysis	-	+	-
DNase	-	+	+
Molecular identification			
T. pyogenes superoxide dismutase A encoding gene *sod*A	+	+	+
T. pyogenes intergenic spacer region (ISR)	+	+	+
Virulence factor encoding genes:			
Pyolysin encoding gene *plo*	+(2)	+	+
Collagen-binding protein encoding gene *cbp*A	-	+	-
Neuraminidase encoding gene *nan*H	-	+	+
Neuraminidase encoding gene *nan*P	+(2)	+	+
Fimbriae encoding gene *fim*A	+(1)	-	+
Fimbriae encoding gene *fim*C	-	+	+
Fimbriae encoding gene *fim*E	+(2)	+	+

The reactions are shown as follows: +, positive reaction; (+), weak reaction; -, negative reaction. The number of positive strains is shown in parentheses after a positive reaction. * = synergistic CAMP-like reactions with staphylococcal β-hemolysin, *Rhodococcus equi, A. haemolyticum* and *A. phocae* as indicator strains.1 = Api-Coryne test system (Biomerieux, Nürtingen, Germany); 2 = tablets containing substrates (Inverness Medical, Köln, Germany); 3 = 4-methylumbelliferyl conjugated substrates (Sigma, Steinheim, Germany).

Gene *plo* expresses the cholesterol dependent pyolysin, a well known virulence factor of *T.* (*A.*) *pyogenes* (Ding and Lämmler, 1996; Billington et al., 1997). Previous studies had indicated that gene *plo* is present in all isolates of this species, suggesting that the determination of gene *plo* could also be used for molecular identification of *T.* (*A.*) *pyogenes* (Ertas et al., 2005; Jost and Billington, 2005; Silva et al., 2008, Ülbegi-Mohyla, et al., 2010; Hijazin, et al., 2011a). As shown previously, *cbp*A, which encodes the collagen-binding protein, appears to be commonly present in isolates from pigs and, comparable to the present study, rarely in isolates of bovine origin, small and wild ruminants and among isolates from various other origins (Santos et al., 2010, Hijazin et al., 2011a). According to Jost and Billington (2005) and Hijazin et al. (2011a) 100 % and 87 % of the *T.* (*A.*) *pyogenes* isolates, respectively carried the neuraminidase encoding gene *nan*H and 64.2 % and 75 %, respectively *nan*P. Both, as proposed by Jost and Billington (2005), seem to play a role in the adhesion of this organism to host epithelial cells. It was of interest that both *T.* (*A.*) *pyogenes* of the present study were negative for *nan*H and positive for *nan*P. According to Jost and Billington (2005) fimbrial encoding genes which show a relation to *Actinomyces naeslundii* type 2 fimbrial subunits also seem to be involved in the adhesion process of *T.* (*A.*) *pyogenes*. Comparable to previous studies (Silva et al., 2008; Santos et al., 2010; Hijazin et al., 2011a) the fimbrial encoding gene *fim*A was found in one isolate and gene *fim*E in both *T.* (*A.*) *pyogenes* isolates of the present study. According to the present results these genes also seem to be useful for genotypic characterization of *T.* (*A.*) *pyogenes* isolated from camels.

The present results give a first detailed description of *T.* (*A.*) *pyogenes* isolates from one-humped camels indicating that bacteria of this origin have similar properties to *T.* (*A.*) *pyogenes* isolated from cattle, pigs or various other animals. Further studies are required to evaluate the significance of these virulence factors for the incidence of lung abscesses in camels and in other animals.

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