Research Article

Phenotypical and Genotypical Properties of an Arcanobacterium pluranimalium Strain Isolated from a Juvenile Giraffe (Giraffa camelopardalis reticulata)

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Received 8 January 2014; Accepted 14 April 2014; Published 30 April 2014

Academic Editor: Daniel A. Feeney

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The present study was designed to characterize phenotypically and genotypically an Arcanobacterium pluranimalium strain (A. pluranimalium 4868) following necropsy from a juvenile giraffe. The species identity could be confirmed by phenotypical investigations and by MALDI-TOF MS analysis, by sequencing the 16S rDNA, pluranimaliumlysin encoding gene pla, and glyceraldehyde-3-phosphate dehydrogenase encoding gene gap with sequence similarities to A. pluranimalium reference strain DSM 13483T of 99.2%, 89.9%, and 99.1%, respectively. To our knowledge, the present study is the first phenotypic and genotypic characterization of an A. pluranimalium strain isolated from a giraffe.

1. Introduction

Genus Arcanobacterium was described by Collins et al. 1982 [1] as a group of facultative anaerobic, asporogenous, and Gram-stain positive rods. According to Yassin et al. (2011) [2], this genus consists of four species, namely, Arcanobacterium haemolyticum, Arcanobacterium hippococcae, Arcanobacterium phocae, and Arcanobacterium pluranimalium. More recently, Arcanobacterium canis and Arcanobacterium phocisimile, two species which were most closely related to A. haemolyticum, were described as novel species of this genus [3, 4].

The original species characterization of A. pluranimalium was performed with two strains isolated from a dead harbour porpoise and a dead fallow deer [5]. In the following years A. pluranimalium could also be isolated from a dog with pyoderma [6], from ovine specimens on 33 occasions, and from a milk sample of a single cow with mastitis [7]. More recently several A. pluranimalium strains recovered from various specimens were identified phenotypically and by using various molecular targets [8].

2. Material and Methods

The present study was focused on the characterization of an A. pluranimalium strain following necropsy from a juvenile giraffe by various phenotypic properties, by MALDI-TOF MS analysis, and genotypically by sequencing 16S rDNA and the A. pluranimalium-specific target genes pla and gap.

The 80.5 kg female giraffe (Giraffa camelopardalis reticulata) of the present study was born in 2013. The giraffe was not accepted by its mother or wet nurse and did not
accept hand rearing attempts and, because of general weakness, was euthanized three days after birth. The subsequent postmortem analysis revealed an acute hyperemia of lung and liver and a focal emphysema of the lung. The acute pneumonia was caused by a bacterial infection associated with aspirated foreign bodies.

Bacteriological investigations yielded the isolation of \textit{A. pluranimalium} and \textit{Escherichia coli}, partly together with coagulase negative staphylococci, \(\alpha\)-haemolytic streptococci, and \textit{Pseudomonas fluorescens} from liver, spleen, kidney, and lung. A moderate to high growth of \textit{E. coli} was generally noted (++, +++; \textit{A. pluranimalium} grew only in low numbers (+). The \textit{A. pluranimalium} strain 4868, originally obtained from the spleen, was used for further studies. The bacterial strain was investigated phenotypically and by MALDI-TOF analysis [6, 9] and genotypically by sequencing of 16S rDNA using universal oligonucleotide primer 16 UNI-L (5'-AGA-GTT-TGA-TCA-GTT-GTC-CAC-GAG-GGA-GGTACA-3') and 16 UNI-R (5'-GTG-GCT-CGG-GGT-TGG-TGT-CAC-3') for amplification, under the following PCR conditions: (x1 (95°C, 600 sec), x30 (95°C, 30 sec, 58°C, 60 sec, 72°C, 60 sec), and using oligonucleotide primer 533-F (5'-TTC-AGT-TGA-TCA-AGT-3') and 907R (5'-GGG-TGA-TGC-CAC-GCA-3') for sequencing.

The strain was also characterized by amplification of the target gene \textit{pla} with the oligonucleotide primer \textit{pla-F}: 5'-GTT-GAT-CTA-CCA-GGA-TTG-AAG-CC-3' and \textit{pla-R}: 5'-TGG-TCG-GGG-TGT-CCA-GGA-GTA-A-3'. Further studies will give information about the constant presence and sequence similarities of both target genes \textit{pla} and \textit{gap}.

Sequencing 16S rDNA, the potentially cytolytic toxin pluranimaliumlysin encoding target gene \textit{pla} and the glyceraldehyde-3-phosphate dehydrogenase encoding target gene \textit{gap} revealed a sequence similarity of 99.2%, 89.9%, and 99.1% to the respective sequences of \textit{A. pluranimalium} DSM 13483\textsuperscript{T}. All three sequences of \textit{A. pluranimalium} 4868 were deposited in GenBank (HG794511, HG423389, and HG423390). A typical dendrogram of the sequencing results of the genes \textit{pla} and \textit{gap} is shown in Figures 1 and 2. Comparable to gene \textit{plo} of \textit{T. pyogenes}, which appeared to be a constant characteristic of all investigated \textit{T. pyogenes} [16–19], \textit{plo} of \textit{A. pluranimalium} seems to also constantly present in all strains of this species and could be used, as described previously [8], and in the present study for molecular identification of \textit{A. pluranimalium}. More recently, Moser et al. 2013 [20] also described \textit{pla} as novel target for molecular identification of this species.

Sequencing of gene \textit{gap} had already been described for molecular identification of staphylococcal species [21] and more recently for identification of an \textit{A. haemolyticus} strain isolated from a donkey [10]. In the present study gene \textit{gap} could also be used as novel target for identification of \textit{A. pluranimalium}. Further studies will give information about the constant presence and sequence similarities of both target genes \textit{pla} and \textit{gap}, respectively.

3. Results and Discussions

\textit{A. pluranimalium} 4868 investigated in the present study was identified by determination of hemolysis and CAMP-like hemolytic reactions, by using a commercial identification system as well as various other phenotypical tests. The CAMP-like hemolytic reactions with \textit{Staphylococcus aureus} \(\beta\)-hemolysin, \textit{Rhodococcus equi}, and \textit{Arcanobacterium haemolyticum} as indicator strains are known as typical characteristics of this species [6, 8, 11].

4. Conclusion

The clinical importance of \textit{A. pluranimalium} of the present study, which was isolated from various organs of the giraffe together with in high number appearing \textit{E. coli}, remains unclear. Since, beside aspiration pneumonia, no other pathological findings could be detected, this might represent the route of infection. However, the isolation of this bacterial species from giraffe and the hitherto described origin harbor porpoise, fallow deer, dog, sheep, and cow emphasizes the species name \textit{A. pluranimalium}. 

\textit{A. pluranimalium} DSM 13483\textsuperscript{T}
Table 1: Biochemical properties of A. pluranimalium 4868 investigated in the present study and A. pluranimalium DSM 13483.

| Biochemical properties | A. pluranimalium 4868 | A. pluranimalium DSM 13483 |
|------------------------|------------------------|-----------------------------|
| Hemolysis on sheep blood agar | + | + |
| CAMP-like reaction with:*  |  |  |
| Staphylococcus aureus β-hemolysin | + | + |
| Streptococcus agalactiae | – | – |
| Rhodococcus equi | + | + |
| Arcanobacterium haemolyticum | + | + |
| Reverse CAMP reaction | – | – |
| Nitrate reduction | –1 | –1 |
| Pyrazinamidase | +1 | +1 |
| Pyrrolidonyl arylamidase | +1 | +1 |
| Alkaline phosphatase | –1 | –1 |
| β-Glucuronidase (β-GUR) | +1,2,3 | +1,2,3 |
| β-Galactosidase (β-GAL) | –1, (+)3 | –1, (+)3 |
| α-Glucosidase (α-GLU) | –1,2,3 | –1,2,3 |
| β-Glucosidase (β-GLU) | +2 | +2 |
| N-Acetyl-β-glucosaminidase (β-NAG) | –1,3 | –1,3 |
| Esculin (β-glucosidase) | (+)3 | +1 |
| Urease | –1 | –1 |
| Gelatine | +1 | +1 |
| Fermentation of: |  |  |
| Glucose | +1 | +1 |
| Ribose | +1 | +1 |
| Xylose | (+)3 | –1 |
| Mannitol | –1 | –1 |
| Maltose | –1 | (+)1 |
| Lactose | –1 | –1 |
| Saccharose | –1 | –1 |
| Glycogen | –1 | –1 |
| α-Mannosidase | –1 | –1 |
| Catalase | – | – |

The reactions are shown as follows: *synergistic CAMP-like reaction with indicator strains; **results mostly obtained from Ulbegi-Mohyla et al., 2010 [6]; +: positive reaction; (+): weak positive reaction; –: negative reaction. *1Api-Coryne test system (Biomerieux, Nürtingen, Germany); 2tablets containing substrates (Rosco Diagnostica A/S, Taastrup, Denmark); 34-methylumbelliferyl conjugated substrates (Sigma, Steinheim, Germany).

Figure 1: Dendrogram of sequences of gene pla of A. pluranimalium 4868 of the present study, three additional A. pluranimalium, and various other cytolytic toxin encoding genes obtained from GenBank.
Conflict of Interests

The authors declare that they have no competing interests. The authors certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this paper.

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Figure 2: Dendrogram of gene gap of A. pluranimalium 4868, reference strain A. pluranimalium DSM 13483T, and various other species of genus Arcanobacterium obtained from GenBank.
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