Identification of a novel 16S rRNA gene variant of Actinomyces funkei from six patients with purulent infections

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Abstract

Little is known about the clinical significance and laboratory diagnosis of Actinomyces funkei. In this report we describe six clinical cases where A. funkei was isolated from purulent, polymicrobial infections. Conventional identification procedures were compared with molecular methods including matrix-assisted laser desorption/ionization time-of-flight mass spectrometry technique. Analysis of the full 16S rRNA gene sequence of the six investigated strains revealed differences from the A. funkei type strain. DNA-DNA hybridization showed that the clinical strains represent a novel 16S rRNA gene variant within the species of A. funkei.

Keywords: Actinomyces funkei, 16S rRNA gene, polymicrobial infection, matrix-assisted laser desorption/ionization time-of-flight, taxonomy

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To date, the genus Actinomyces comprises 38 described species of which many have been reported from human sources [1]. Apart from the ‘classical’ actinomycosis involving abscesses in the mouth, lungs or gastrointestinal tract and often forming polymicrobial infection, Actinomyces species have been implicated in abscess formation at other body sites, infected atheroma, infection of skin structures, endophthalmitis and bacteraemia including endocarditis [2,3]. Actinomyces funkei is a species first described in 2001 [4]. To date, there is only one clinical report on A. funkei in the literature describing endocarditis in an intravenous drug abuser [5]. As little is known of the natural habitat and clinical significance of this species, we present six clinical cases of infection with A. funkei as well as detailed data on microbiological identification including biochemistry, full 16S rRNA gene sequencing, and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry analysis.

During a period from 2007 to 2011, we isolated six strains of A. funkei from patients of the University Hospital Basel, Switzerland. Actinomyces funkei was isolated from three abscesses, one biopsy and two superficial wound swabs; it was always isolated together with concomitant bacteria and yeasts. Microbiological and clinical data of the six patients from whom A. funkei was isolated are presented in Table 1. After 24–48 h of aerobic as well as anaerobic incubation, small, greyish, catalase-negative colonies appeared on Columbia agar supplemented with 5% sheep blood (BD Diagnostic Systems, Allschwil, Switzerland). Gram-stain revealed irregular, slightly branched Gram-positive rods. The biochemical characterization of aerobic Gram-positive rods was performed with API Coryne™ (bioMérieux, Geneva, Switzerland). Two strains were identified as Arcanobacterium haemolyticum with scores consistent with very good (API code 2530761) and good (API code 3130761) identification profiles; the remaining four strains were identified with unacceptable identification profiles (API code 0130721 in two strains; 2130521; 0110521). Three strains were tested with MALDI-TOF mass spectrometry (MALDI Biotyper, Bruker Daltonics GmbH, Bremen, Germany) by using normal, short extraction and full extraction protocols as described elsewhere [6]. MALDI-TOF identified all three strains as A. funkei as first choice. Normal and short extraction protocols generated average scores of 1.949 and 1.946, respectively, which is in the range of scores indicating correct genus identification. The average of the scores produced by using the full extraction protocol was 2.158, which is consistent with probable species identification. Routine partial 16S rRNA gene amplification and sequencing were performed by using the Fast MicroSeq® 500 16S rRNA gene Bacterial Identification Sequencing Kit (Applied Biosystems, Rotkreuz, Switzerland). Comparison of partial 16S rRNA gene sequences with the MicroSeqID® 500 software (version 2.1.) database revealed 94.1% identity to Actinomyces turicensis. Comparison with the GenBank database (National Center for Biotechnology Information, Bethesda, MD, USA) showed 99.6% identity with the A. funkei CCUG 42773T, whereas the next related species was Actinomyces turicensis with 94.3% identity. The alignment of the entire 16S rRNA gene sequence of all six A. funkei strains revealed 100% identity within the strains (MEGAalign 6.1 sequence analysis software: Lasergene, DNASTar, Madison,
Interestingly, when full 16S rRNA gene sequences of the investigated strains of this study were aligned with the A. funkei type strain CCUG 42773T, 12 polymorphisms were detected (Table 2). This corresponds to 99.1% identity, which could indicate a novel Actinomyces species based on the cut-off value for species identification of aerobic Actinomyces (≥ 99.6% identity) from CLSI guidelines [7]. Therefore, to investigate the overall genetic similarity between the investigated strains and the A. funkei type strain, we performed DNA–DNA hybridization according to the method described previously [8], which resulted in 95–100% similarity between four investigated strains and the A. funkei type strain CCUG 42773T. This indicates that our strains represent a novel 16S rRNA gene variant within the A. funkei species.

**Actinomyces funkei** belongs to the group of recently described, aero-tolerant Actinomyces species. Six strains described in our study have been associated with abscess formation and other purulent infections (Table 1). Other *Actinomyces* species are known to be members of the normal flora of the skin and mucous membranes [3,4,9], so this could also be the case for A. funkei. The infections with this species may therefore occur when the skin or mucosal surface is damaged. In patient number 3, A. funkei was isolated from a liver abscess where an intestinal focus of infection was suspected following diverticulitis surgery. Association of gastrointestinal infections including liver abscesses due to *Actinomyces* sp. with abdominal surgery, intra-abdominal inflammation, or the presence of foreign bodies is well documented in the literature [9–11]. In all six investigated samples, numerous concomitant bacteria were isolated together with A. funkei, which is congruent with the previous reports on *Actinomyces* species being frequently found in polymicrobial infections [9,12]. On the other hand, occurrence in mixed cultures could mean that this species is frequently overlooked in the routine evaluation of microbiological cultures. Identification of this species using conventional biochemical tests can be misleading and false because A. funkei is not deposited in the Api Coryne™ database. Ng et al. [13] have reported that MALDI-TOF mass spectrometry is a promising tool for identification of different aero-tolerant *Actinomyces* spp. including A. funkei, but improvement of the database may increase the confidence of species identification. This observation is further confirmed in our study where the highest scores were obtained by testing the A. funkei strains with a full extraction protocol. Sequencing of the 16S rRNA gene proved to be a reliable tool for identification of this bacterium, but because of its complexity and higher costs, it may not be available in all laboratories. Bearing in mind the great genetic heterogeneity of the genus *Actinomyces* [14], the 12 polymorphisms between the investigated strains and the A. funkei type strain (Table 2) brought up the question of discovery of a new *Actinomyces* species. However, DNA–DNA hybridization confirmed that our investigated strains represent a 16S rRNA gene variant of A. funkei.

### TABLE 1. Microbiological and clinical findings in patients from whom *Actinomyces funkei* was isolated

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Specimen type</th>
<th>Gram stain (amount)</th>
<th>Culture (amount)</th>
<th>Clinical presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65/F</td>
<td>Superficial wound swab (ischial)</td>
<td>Leucocytes (+++), Gram-positive rods (+++)</td>
<td>A. funkei (+++), enterobacteriaceae (+), yeasts (+)</td>
<td>Wound infection following hysterectomy</td>
</tr>
<tr>
<td>2</td>
<td>26/F</td>
<td>Abcess (vulva)</td>
<td>Leucocytes (+++), Gram-negative pleomorphic rods (+++)</td>
<td>A. funkei (+++), anaerobic mixed flora (+++)</td>
<td>Vulvar abscess</td>
</tr>
<tr>
<td>3</td>
<td>59/M</td>
<td>Abscess (liver)</td>
<td>Leucocytes (+++), Gram-positive cocci (+++), Gram-negative rods (+)</td>
<td>A. funkei (+++), Bacteroides fragilis-group (+++), Escherichia coli (+), Morganella morganii (+), Streptococcus anginosus-group (+), Gram-positive anaerobic cocci (+)</td>
<td>Multiple liver abscesses, peritonitis and diverticulitis, polymicrobial sepsisemia</td>
</tr>
<tr>
<td>4</td>
<td>47/M</td>
<td>Abscess (gluteal)</td>
<td>Leucocytes (+), erythrocytes (+++), cell debris (+), Gram-negative cocci (+)</td>
<td>A. funkei (+), Staphylococcus lugdunensis (+), Anaerobic mixed flora (+)</td>
<td>Pilonidal abscess</td>
</tr>
<tr>
<td>5</td>
<td>66/M</td>
<td>Superficial wound swab (scrotum)</td>
<td>Leucocytes (+++), epithelial cells (+), Gram-positive cocci (+)</td>
<td>A. funkei (+), CoNS® (+)</td>
<td>Abdominocrotal wound infection after surgery</td>
</tr>
<tr>
<td>6</td>
<td>81/M</td>
<td>Biopsy (ischial pressure ulcer)</td>
<td>Leucocytes (+), erythrocytes (+++), Gram-positive cocci (+)</td>
<td>A. funkei (+), Streptococcus anginosus-group (+), anaerobic mixed flora (+)</td>
<td>Ischial pressure ulcer with severe sepsis due to Streptococcus anginosus and Bacteroides fragilis</td>
</tr>
</tbody>
</table>

M, male; F, female; CoNS, coagulase-negative staphylococci; +, few; ++ moderate; +++, numerous.

### TABLE 2. Position of 12 polymorphisms within the 16S rRNA gene of six *Actinomyces funkei* strains compared with *Actinomyces funkei* type strain CCUG 42773T (Accession No. NR 028960.1)

<table>
<thead>
<tr>
<th>Nucleotide position in the 16S rRNA gene</th>
<th>276</th>
<th>297</th>
<th>515</th>
<th>520</th>
<th>574</th>
<th>786</th>
<th>797</th>
<th>798</th>
<th>947</th>
<th>951</th>
<th>952</th>
<th>963</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. funkei CCUG 42773T</td>
<td>C</td>
<td>T</td>
<td>A</td>
<td>T</td>
<td>T</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>A. funkei strains 1-6</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
The complete 16S rRNA gene sequences from the six strains described in this study have been assigned to the GenBank database with accession numbers JQ031122.1, JQ031123.1, JQ031124.1, JQ031125.1, HQ906496.2 and HQ906497.1. Three strains have been deposited at the CCUG Culture Collection in Göteborg, Sweden, under the accession numbers CCUG 61725, CCUG 61726 and CCUG 61727.

Parts of this study have been presented at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), held in London from 31 March to 3 April 2012.

Transparency Declaration

None of the authors have to declare conflict of interest.

References