NOTE

***Actinomyces funkei* sp. nov., isolated from human clinical specimens**Paul A. Lawson,¹ Natalia Nikolaitchouk,² Enevold Falsen,² Katarina Westling³ and Matthew D. Collins¹¹ Department of Food Science and Technology, Whiteknights, PO Box 226, Reading RG6 6AP, UK² Culture Collection, Department of Clinical Bacteriology, University of Göteborg, Sweden³ Department of Infectious Diseases, Huddinge University Hospital, Karolinska Institutet, Sweden

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Three strains of a previously undescribed *Actinomyces*-like bacterium were isolated from human clinical specimens. Phenotypic studies indicated that the strains were members of the genus *Actinomyces* and were presumptively identified as *Actinomyces turicensis*. Comparative 16S rRNA gene sequencing studies showed that although the bacterium is phylogenetically closely related to *Actinomyces turicensis*, it nevertheless constitutes a new sub-line within the genus *Actinomyces*. Based on phenotypic and molecular chemical and molecular genetic evidence, it is proposed that the unknown *Actinomyces*-like bacterium from human clinical specimens be classified as *Actinomyces funkei* sp. nov. The type strain of *Actinomyces funkei* is CCUG 42773^T (= CIP 106713^T).

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Actinomyces species predominantly belong to the facultatively anaerobic indigenous microflora of human and animal mucous membranes (i.e. oral cavity, intestinal tract and female genital tract). Many *Actinomyces* species are established pathogens of man and animals. Some species cause the clinically well-defined inflammatory disease termed actinomycosis, whereas some others may be involved aetiologically in a variety of diseases such as pharyngitis, urethritis, cutaneous or subcutaneous suppurations and even endocarditis in humans. In addition, several *Actinomyces* species play a role in the complex aetiology of coronal and root caries and surface periodontal diseases. Despite the importance of *Actinomyces* species, it is only in recent years that clinical microbiologists have begun to fully appreciate the enormous diversity of these organisms from human/animal sources, and in the past few years a plethora of new *Actinomyces* and related taxa have been described (e.g. Funke *et al.*, 1994, 1997; Nikolaitchouk *et al.*, 2000; Pascual Ramos *et al.*, 1997; Pascual *et al.*, 1999; Wüst *et al.*, 1995) and the number of validated species has almost doubled. In this article, we report the characteristics of three unusual *Actinomyces*-like isolates recovered from human clinical sources.

Strain CCUG 42773^T was isolated from a 40-year-old female intravenous drug user who had a history of *Staphylococcus aureus* endocarditis of the tricuspid

valve 6 months earlier. She received cefuroxime as part of antibiotic treatment. Three out of four blood cultures taken yielded a Gram-positive rod that was tentatively identified as *Actinomyces turicensis*. Strain CCUG 39151 was recovered from human sternum (Göteborg, Sweden) and strain CCUG 34685 was isolated from a human abdominal incision (Washington, USA). Both strains were deposited in the Culture Collection of the University of Göteborg as *Actinomyces turicensis*. The *Actinomyces turicensis*-like isolates were cultured on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C, in air plus 5% CO₂. The strains were biochemically characterized by using the API rapid ID32Strep, API CORYNE and API ZYM systems according to the manufacturer's instructions (API bioMérieux). PAGE analysis of whole-cell proteins was performed as described by Pot *et al.* (1994). Generated profiles were compared with a comprehensive database maintained by the CCUG. The 16S rRNA genes of the isolates were amplified by PCR and directly sequenced using a Taq Dye-Deoxy Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). A phylogenetic tree was constructed according to the neighbour-joining method and the stability of the groupings was estimated by bootstrap analysis (Felsenstein, 1989).

The three isolates consisted of Gram-positive, slender, straight to slightly curved rods, some of which exhibited branching. Cells were non-acid-fast and non-

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 42773^T is AJ404889.

Fig. 1. Unrooted tree showing the phylogenetic relationships of *Actinomyces funkei* sp. nov. and some other closely related *Actinomyces* species. The tree, constructed using the neighbour-joining method, was based on a comparison of approximately 1327 nucleotides. Bootstrap values, expressed as a percentage of 500 replications, are given at branching points.

spore-forming. The strains grew under aerobic and anaerobic conditions and were catalase-negative. When grown on Columbia blood agar for 24 h, the colonies were small (< 1 mm in diameter), non-haemolytic and grey in colour. Older cultures displayed fried-egg-type colony morphology. Using API systems, the isolates phenotypically closely resembled each other, producing acid from glucose, sucrose and D-xylose, but not from L-arabinose, D-arabitol, cyclo-dextrin, mannitol, melibiose, melezitose, methyl β -D-glucopyranoside, glycogen, pullulan, raffinose, sorbitol, tagatose or trehalose. Acid production from maltose and ribose was found to be variable. Different results were obtained for acid production from lactose using the API CORYNE and API rapid ID32Strep tests. Lactose fermentation was positive with the API CORYNE test kit but negative using the API rapid ID32Strep system. Acid phosphatase, alanine phenylalanine proline arylamidase, alkaline phosphatase, cystine arylamidase (weak reaction), α -glucosidase and leucine arylamidase were produced by the isolates but tests for arginine dihydrolase, β -glucosidase, chymotrypsin, esterase C4, ester lipase C8, α -fucosidase, α -galactosidase, β -glucuronidase, glycyl tryptophan arylamidase, α -mannosidase, β -mannosidase, lipase C14, pyrrolidonyl arylamidase, pyroglutamic acid arylamidase, trypsin, valine arylamidase and urease were negative. Variable reactions were observed for β -galactosidase and pyrazinamidase. The production of *N*-acetyl- β -glucosaminidase was detected using the API CORYNE and API ZYM test strips but was negative with the API rapid ID32 Strep kit. All of the isolates hydrolysed hippurate but not aesculin or gelatin. They gave a negative Voges-Proskauer test. Two of the three strains reduced nitrate. The cellular morphology and biochemical reactions of the isolates were consistent with their assignment to the genus *Actinomyces* but did not appear to correspond to any described species. The results of a comparative analysis of whole-cell protein profiles of the unknown strains and other *Actinomyces* species by SDS-PAGE showed that the three isolates were highly related to each other and were distinct from recognized *Actinomyces* species.

The three isolates clustered together, forming a distinct group with a within-group correlation level of 70 % or more. *Actinomyces hyovaginalis* was the nearest neighbour to the unknown isolates, joining the latter at a correlation level of less than 55 % (data not shown). To ascertain the phylogenetic position of the unknown bacterium, comparative 16S rRNA gene sequencing was conducted. The almost complete gene sequences of two of the isolates (CCUG 42773^T and CCUG 34685) were determined and pairwise analysis showed these to be identical (1400 nucleotide bases compared). A stretch of 850 bases was also determined for CCUG 39151, which was also found to possess 100 % sequence similarity with the other two strains. Sequence database searches confirmed the unknown bacterium was most closely related to species of the genus *Actinomyces*. The results of neighbour-joining analysis showed that *Actinomyces turicensis* was the nearest relative of the unknown bacterium (Fig. 1), displaying 96.6 % sequence similarity. However, bootstrap re-sampling showed that the association between the unknown bacterium and *Actinomyces turicensis* was not statistically significant (value 58 % for 500 tree replications).

It is apparent from both phenotypic and phylogenetic evidence that the isolates from human clinical specimens represent a hitherto unknown *Actinomyces* species. All three isolates were received by the CCUG as tentative *Actinomyces turicensis*. 16S rRNA gene sequencing clearly shows that *Actinomyces turicensis* is in fact the closest phylogenetic relative of the unknown clinical isolates, although a sequence divergence of 3.4 % demonstrates that the latter represent a distinct species. *Actinomyces turicensis* was proposed by Wüst *et al.* (1995) for some *Actinomyces* (*Arcanobacterium*) *pyogenes*-like organisms recovered from mixed cultures from a variety of human infections. Many isolates previously resembling and/or presumptively identified as *Gardnerella vaginalis* and *Arcanobacterium* species have subsequently been shown to correspond to *Actinomyces turicensis* (Vandamme *et al.*, 1998). Since the original description of *Actinomyces*

turicensis, this species has been isolated from a wide range of clinical samples, in some cases as the sole isolate, indicative of clinical relevance (Vandamme *et al.*, 1998; Sabbe *et al.*, 1999). It seems unlikely that the unknown bacterium reported here has in the past been confused or misidentified with *Actinomyces turicensis* given their distinct phenotypic traits. The widely used API CORYNE test system is of particular value in distinguishing the unknown bacterium from *Actinomyces turicensis*. This test system has been shown to give very stable codes for *Actinomyces turicensis* [viz. 0(2)01070(2)1], which differ markedly from the codes obtained for the three unidentified clinical isolates (viz. 0130761, one strain; 3530761, two strains). The distinct biochemical characteristics of the isolates together with molecular chemical and molecular genetic findings clearly demonstrate that the unknown bacterium merits classification as a new species, for which the name *Actinomyces funkei* is proposed. The description of *Actinomyces funkei* should facilitate the identification of this bacterium in the clinical laboratory, thereby improving information on its association with various clinical conditions and its possible pathological significance.

Description of *Actinomyces funkei* sp. nov.

Actinomyces funkei (fun.ke.i. N.L. gen. n. *funkei* of Funke, to honour Guido Funke, a contemporary German microbiologist, for his contributions to the clinical microbiology of *Actinobacteria*).

Cells are Gram-positive, slender, straight to slightly curved rods, some of which exhibit branching, which are non-acid-fast and non-spore-forming. Facultatively anaerobic and catalase-negative. When grown on Columbia blood agar for 24 h, colonies are small (< 1 mm in diameter), non-haemolytic and grey in colour. Using API systems, acid is produced from glucose, sucrose and D-xylose, but not from L-arabinose, D-arabitol, cyclodextrin, mannitol, melibiose, melezitose, methyl β -D-glucopyranoside, glycogen, pullulan, raffinose, sorbitol, tagatose or trehalose. Acid production from lactose, maltose and ribose is variable. Acid phosphatase, alanine phenylalanine proline arylamidase, alkaline phosphatase, cystine arylamidase (weak reaction), α -glucosidase and leucine arylamidase are produced. Arginine dihydrolase, β -glucosidase, chymotrypsin, esterase C4, ester lipase C8, α -fucosidase, α -galactosidase, β -glucuronidase, glycyl tryptophan arylamidase, α -mannosidase, β -mannosidase, lipase C14, pyroglutamic acid arylamidase, pyrrolidonyl arylamidase, trypsin, valine arylamidase and urease are negative. N-Acetyl- β -glucosaminidase, β -galactosidase and pyrazinamidase may or may not be produced. Hippurate is hydrolysed

but not aesculin or gelatin. Acetoin is not produced. Nitrate reduction is variable. Isolated from human clinical specimens. Habitat is not known. The type strain is CCUG 42773^T (= CIP 106713^T).

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