Actinomyces oricola sp. nov., from a human dental abscess

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A previously undescribed *Actinomyces*-like bacterium was isolated from a human dental abscess. Based on its cellular morphology and the results of biochemical testing the organism was tentatively identified as a member of the genus *Actinomyces*, but it did not correspond to any currently recognized species of this genus. Comparative 16S rRNA gene sequencing studies showed the bacterium represents a hitherto unknown subline within the genus *Actinomyces*, clustering within a group of species, which includes *Actinomyces bovis*, the type species of the genus. Based on biochemical and molecular phylogenetic evidence, it is proposed that the unknown organism recovered from a dental abscess be classified as a new species, *Actinomyces oricola* sp. nov. The type strain of *Actinomyces oricola* is R5292^T (=CCUG 46090^T = CIP 107639^T).

The genus *Actinomyces* encompasses a taxonomically heterogeneous collection of anaerobic and aerotolerant, non-spore-forming, non-acid-fast, Gram-positive rod-shaped organisms within the *Actinobacteria*. In the last decade the taxonomy of this complex group has undergone much improvement, in part due to an increased interest in the identification of these organisms, particularly from the clinical environment (Funke *et al.*, 1997b; Hall *et al.*, 1999, 2001). The use of molecular taxonomic methods [such as 16S rRNA sequencing, amplified 16S rDNA restriction analysis (ARDRA) and protein profiling] have done much to aid the identification of *Actinomyces*-like organisms and the recognition of novel species (e.g. Collins *et al.*, 2000; Funke *et al.*, 1994, 1997a; Hall *et al.*, 1999, 2001; Lawson *et al.*, 2001; Nikolaitchouk *et al.*, 2000; Pascual *et al.*, 1997; Wüst *et al.*, 1995). However, despite marked improvements in the identification of many *Actinomyces*-like organisms, especially from human sources, it is evident that many taxonomically problematic organisms remain (Hall *et al.*, 1999, 2001). It is important that atypical or unusual *Actinomyces*-like organisms are characterized to increase knowledge of their natural habitats and possible clinical associations and prevalence. In the course of an ongoing study exploring the diversity of *Actinomyces*-like

organisms from human sources, we have characterized an organism, which although phenotypically consistent with the genus *Actinomyces*, does not appear to correspond to any recognized species. Based on the results of a polyphasic taxonomic study, we describe yet another new species of the *Actinomyces* genus, *Actinomyces oricola* sp. nov.

The bacterial isolate R5292^T was isolated from a dental abscess of a male patient in Bury and was referred to the Anaerobe Reference Unit, PHLS, University Hospital of Wales, for identification. No further clinical information is known. For biochemical testing the strain was cultured on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C, incubated anaerobically. The strain was biochemically characterized by using both conventional tests (Phillips, 1976) and the commercially available API rapid ID32Strep, API rapid ID32A and API Coryne systems, according to the manufacturer's instructions (API bioMérieux). Volatile and non-volatile end products of glucose metabolism were detected by gas-liquid chromatography (Holdeman *et al.*, 1977). PAGE analysis of whole-cell proteins was performed as described by Pot *et al.* (1994). Long-chain cellular fatty acids were examined using the MIDI system. ARDRAs were performed using *Hae*III and *Hpa*II as described previously (Hall *et al.*, 1999). The 16S rDNA of the isolate was 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). Phylogenetic analyses were performed as described by Collins *et al.* (2000).

Abbreviations: ARDRA, amplified 16S rDNA restriction analysis; CCUG, Culture Collection of the University of Göteborg; CIP, Collection of Bacterial Strains of Institut Pasteur.

The GenBank accession number for the 16S rRNA sequence of CCUG 46090^T is AJ507295.

The unidentified organism consisted of Gram-positive, rod-shaped cells, some of which displayed branching, and filaments were observed. Cells were non-acid-fast, non-spore-forming and non-motile. Colonies after 48 h anaerobic incubation on Fastidious Anaerobe Agar with 5% horse blood were pin-point, breadcrumb-like, white and non-haemolytic. The organism was catalase-negative and facultatively anaerobic, but grew best under anaerobic conditions. The end products of glucose metabolism were acetic acid, lactic acid and succinic acid. Using conventional biochemical testing, the organism produced acid from amygdalin (weak), cellobiose, fructose, glucose, raffinose (weak), salicin, sucrose and trehalose, but not from arabinose or lactose. The organism hydrolysed aesculin, but failed to hydrolyse gelatin or starch. It was lipase-, lecithinase- and urease-negative, and did not produce indole. Employing commercial API biochemical kits the strain was unidentified. Using the API Rapid 32Strep system, the organism was relatively inactive, displaying activity for α -galactosidase, β -glucosidase and alanine phenylalanine proline arylamidase (weak reaction). All other tests in this gallery gave negative reactions, producing a numerical code of 220020 00000. Using the API Coryne system, acid was formed from glucose, maltose and sucrose, but not from any of the other carbohydrates. The organism hydrolysed aesculin and displayed activity for alkaline phosphatase, α -glucosidase, β -galactosidase and pyrazinamidase. All other tests in the API Coryne kit were negative, giving a numerical profile of 2550121. With the API Rapid ID 32A system, the organism produced acid from mannose and raffinose and gave positive reactions for alkaline phosphatase, alanine arylamidase, arginine arylamidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, glycine arylamidase, proline arylamidase, leucyl glycine arylamidase, leucine arylamidase, phenylalanine arylamidase and tyrosine arylamidase. All other tests were negative, giving an API Rapid ID 32A numerical profile of 4516473700.

The cellular morphology and general physiological and biochemical reactions of the organism were consistent with its tentative assignment to the genus *Actinomyces*, but it

did not appear to correspond to any recognized species of this genus. An examination of the long-chain cellular fatty acids of the unidentified organism revealed the presence of straight-chain saturated and monounsaturated acids: C12:0 (0.7%), C14:0 (1.1%), C16:0 (44.0%), C16:1 (2.3%), C18:0 (14%), C18:1 *cis*9 (37%) and C20:1 *cis*11 (0.9%). This fatty acid composition reinforces the relationship of the unknown organism to the genus *Actinomyces*. To investigate the possible association of the unknown bacterium with *Actinomyces* species, its whole-cell protein profile was compared with those of known *Actinomyces* species. Comparative analysis of whole-cell protein profiles showed the unknown organism was distinct from all currently defined species of this genus (data not shown). A dendrogram based on a subset of species depicting comparative protein analysis of the unidentified species and its closest relatives is shown in Fig. 1. *Actinomyces howellii* (CCUG 32757^T, CCUG 35943) displayed the closest similarity to the unidentified clinical organism, joining the latter at about 73% similarity. Other species displayed much lower levels of similarity (Fig. 1). To further investigate the genetic relatedness of the unidentified organism to *Actinomyces* species, ARDRA was performed. The unknown organism produced a unique 16S rDNA restriction pattern with *Hae*III and *Hpa*II (profile 019/017) and was distinct from the profiles derived from the analysis of over 400 *Actinomyces* strains (Hall *et al.*, 2001).

To determine the phylogenetic relationships of the unidentified organism, its almost complete 16S rRNA gene sequence (>1400 nt) was determined. Sequence database searches confirmed the unknown isolate was most closely related to species of the genus *Actinomyces*. Highest sequence similarity values were shown with *Actinomyces naeslundii* NCTC 10301^T (94.2%), *Actinomyces bowdenii* CCUG 37421^T (94.0%), *Actinomyces viscosus* NCTC 10951^T (93.9%), *Actinomyces israelii* CIP 103259^T (93.9%), *Actinomyces howellii* NCTC 11636^T (93.7%), *Actinomyces denticolens* NCTC 11490^T (93.7%) and phylogenetically related organisms. Treeing analysis further demonstrated the placement of the unidentified bacterium within the genus *Actinomyces*, with the novel

Fig. 1. Similarity dendrogram based on whole-cell protein patterns of *Actinomyces oricola* sp. nov. and closely related species. Levels of correlation are expressed as percentages of similarity for convenience.

Fig. 2. Unrooted tree showing the phylogenetic relationships of *Actinomyces oricola* sp. nov. and other species of the genus *Actinomyces* and related taxa. The tree constructed using the neighbour-joining method was based on a comparison of approximately 1327 nt. Bootstrap values, expressed as a percentage of 500 replications, are given at branching points.

bacterium forming a distinct subline within a small subcluster of species which includes *Actinomyces bovis*, the type species of the genus *Actinomyces* (Fig. 2).

Based upon both phenotypic and molecular phylogenetic studies it is clear that the unidentified Gram-positive, catalase-negative, rod-shaped organism recovered from a dental abscess represents a hitherto unknown *Actinomyces* species from human sources. Phylogenetically, the unknown bacterium forms a distinct subline within the *Actinomyces* genus and displays an affinity with a cluster of species, including *Actinomyces bovis*, *Actinomyces catuli*, *Actinomyces naeslundii*, *Actinomyces bowdenii*, *Actinomyces viscosus*, *Actinomyces israelii*, *Actinomyces gerencseriae*, *Actinomyces howellii*, *Actinomyces denticolens*, *Actinomyces radidentis*, *Actinomyces slackii* and *Actinomyces urogenitalis*. Bootstrap resampling, however, showed the unknown bacterium did not share a statistically significant association with any individual member of this rRNA subcluster. Sequence divergence values of approximately 5.6–7.6 % reinforced the separateness of the unidentified oral bacterium from all currently recognized members of this subcluster. Although there is no precise correlation between percentage 16S rRNA sequence divergence values and species delineation, it is now generally accepted that organisms displaying values of 3 % or more do not belong to the same species (Stackebrandt & Goebel, 1994). The observed >5 % sequence divergence between the unidentified clinical isolate and all currently defined *Actinomyces* species is therefore consistent with separate species status.

Strong support for the separateness of the unknown bacterium also comes from phenotypic evidence. In particular, comparative whole-cell protein profiling shows the unidentified organism is distinct from all defined *Actinomyces* species. Furthermore the unidentified bacterium possesses a very characteristic biochemical profile, which serves to distinguish it from all currently described *Actinomyces* species. The unidentified organism can be readily distinguished from its nearest phylogenetic relatives using commercial API Rapid ID 32Strep and API Rapid ID 32A kits. In particular, the novel organism differs markedly from *Actinomyces bowdenii*, *Actinomyces catuli*, *Actinomyces denticolens*, *Actinomyces israelii*, *Actinomyces howellii*, *Actinomyces naeslundii*, *Actinomyces radidentis*, *Actinomyces slackii*, *Actinomyces viscosus* and *Actinomyces urogenitalis* in

producing acid from a far smaller range of sugars. In addition, the unknown organism can be readily distinguished from *Actinomyces bovis* by producing α -galactosidase and α -glucosidase. By contrast, *Actinomyces bovis* gives negative results for these tests.

Therefore, based on the distinct phenotypic characteristics of the unidentified rod-shaped bacterium and molecular phylogenetic evidence, we consider it warrants classification as a new species of the *Actinomyces* genus, for which the name *Actinomyces oricola* sp. nov. is proposed. Although only a single strain of *Actinomyces oricola* is currently known, we consider the formal description of this species together with biochemical criteria to aid its identification, will in the future facilitate its recognition in the routine laboratory, thereby permitting the recovery of additional strains and an evaluation of its distribution, clinical prevalence and possible significance. Tests which are useful in distinguishing *Actinomyces oricola* from its nearest phylogenetic relatives are shown in Table 1.

Description of *Actinomyces oricola* sp. nov.

Actinomyces oricola (o.ri'co.la. L. n. *os*, *oris* mouth; L. masc. suffix *cola* inhabitant of; N.L. masc. n. *oricola* inhabitant of the mouth).

Gram-positive, rod-shaped cells, some of which display branching and filaments may be observed. Cells are non-acid-fast, non-spore-forming and non-motile. Colonies after 48 h anaerobic incubation on Fastidious Anaerobe Agar with 5 % horse blood are pin-point, breadcrumb-like, white and non-haemolytic. Catalase-negative and facultatively anaerobic, but grows best under anaerobic conditions. The end products of glucose metabolism are acetic, lactic and succinic acids. Using conventional tests, acid is produced from amygdalin (weak), cellobiose, fructose, glucose, raffinose (weak), salicin, sucrose and trehalose, but not from arabinose or lactose. Aesculin is hydrolysed, but gelatin and starch are not. Lipase, lecithinase and urease are not detected and indole is not produced. Using commercial API systems, acid is produced from glucose, but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, mannitol, melibiose, melizitol, methyl- β -D-glucopyranoside, pullulan, D-ribose, sorbitol, tagatose, trehalose or D-xylose. Acid may or may not be produced from maltose, sucrose and raffinose,

Table 1. Tests useful in distinguishing *Actinomyces oricola* sp. nov. from its nearest relatives using the API Rapid ID32Strep system

1, *Actinomyces bowdenii* (n=5); 2, *Actinomyces denticolens* (n=15); 3, *Actinomyces gerencseriae* (n=4); 4, *Actinomyces howellii* (n=2); 5, *Actinomyces naeslundii* (n=20); 6, *Actinomyces oricola* (n=1); 7, *Actinomyces radicidentis* (n=3); 8, *Actinomyces slackii* (n=4); 9, *Actinomyces viscosus* (n=20); 10, *Actinomyces urogenitalis* (n=5). MBDG, Methyl- β -D-glucopyranoside; ADH, arginine dihydrolase; APPA, alanine phenylalanine proline arylamidase; β -Gal, β -Galactosidase; +, >90% give positive reactions; -, <10% give positive reactions; v, variable.

Characteristic	1	2	3	4	5	6	7	8	9	10
Acid from:										
D-Arabitol	-	-	-	-	-	-	-	-	-	+
Lactose	+	+	v	+	v	-	+	+	v	+
Melibiose	v	+	v	+	+	-	+	v	+	+
Melezitose	+	v	-	+	-	-	+	-	-	+
Maltose	+	+	v	+	+	-	+	+	+	+
Sucrose	+	+	v	+	+	-	+	+	+	+
Trehalose	+	+	v	+	v	-	+	+	-	+
MBDG	+	+	-	+	v	-	+	+	v	+
Activity for:										
ADH	-	-	v	+	-	-	-	-	-	+
APPA	+	v	-	+	-	+	+	-	+	+
β -Gal	+	+	+	+	+	-	+	+	+	+

depending on the test system used. Aesculin is hydrolysed, but gelatin and hippurate are not. Indole is not produced and nitrate is not reduced. Alanine arylamidase, alanine phenylalanine proline arylamidase, arginine arylamidase, α -galactosidase, α -glucosidase, β -glucosidase, glycine arylamidase, proline arylamidase, pyrazinamidase, leucyl glycine arylamidase, leucine arylamidase, phenyl alanine arylamidase and tyrosine arylamidase are detected. Activity for alkaline phosphatase and β -galactosidase may or may not be detected, depending on the test system used. No activity is detected for α -arabinosidase, arginine dihydrolase, α -fucosidase, β -galactosidase-6-phosphate, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, β -glucuronidase, glycyl tryptophane arylamidase, β -mannosidase, pyroglutamic acid arylamidase, N-acetyl- β -glucosaminidase, histidine arylamidase, serine arylamidase or urease. Voges-Proskauer-negative. The major long-chain fatty acids are C16:0, C18:0 and C18:1 ω 9c. Isolated from human dental abscess. Habitat is not known. Type strain is R5292^T (=CCUG 46090^T=CIP 107639^T).

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