Actinomyces nasicola sp. nov., isolated from a human nose

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A previously undescribed facultatively anaerobic, catalase-negative, *Actinomyces*-like bacterium was isolated from the nose of a human. On the basis of its cellular morphology and the results of biochemical testing, the micro-organism was tentatively identified as a member of the genus *Actinomyces*, but it did not correspond to any currently recognized species. Comparative 16S rRNA gene sequencing studies showed the bacterium to be a hitherto unknown subline within the genus *Actinomyces*, displaying sequence divergence values of more than 6% with respect to recognized species of the genus. On the basis of biochemical, molecular chemical and molecular phylogenetic evidence, it is proposed that the unknown organism, strain R2014^T (= CCUG 46092^T = CIP 107668^T), be classified as the type strain of a novel species, *Actinomyces nasicola* sp. nov.

In recent years, the genus *Actinomyces* has undergone considerable expansion. In the 2nd edition of *The Prokaryotes*, only 13 species were listed in the genus (Schaal, 1992). However, in the past decade, the genus has increased dramatically in size and at the time of writing comprises 28 species. The vast majority of these new additions to the genus have originated from human clinical specimens, where they occur as mucosal contaminants, whilst some others possibly represent unknown opportunistic pathogens (e.g. Collins *et al.*, 2000; Funke *et al.*, 1994, 1997; Hall *et al.*, 2002; Lawson *et al.*, 2001; Nikolaitchouk *et al.*, 2000; Pascual Ramos *et al.*, 1997; Wüst *et al.*, 1995). Despite this rapid increase in the number of recognized *Actinomyces* species, it is clear that much novel diversity remains to be discovered, particularly from human sources (Hall *et al.*, 1999, 2001). For example, Hall *et al.* (2001), using amplified 16S rDNA restriction analysis to identify clinical *Actinomyces* isolates, reported that many of the organisms exhibited genetic profiles that did not correspond to recognized species, raising the possibility that much novel diversity remains to be elucidated from humans. In the course of an ongoing study of taxonomically problematic actinobacteria, we have characterized an unknown catalase-negative, facultatively anaerobic, *Actinomyces*-like micro-organism from the nose of a patient. On the basis of the results of a polyphasic taxonomic study, we describe yet

another novel species of the genus *Actinomyces*, *Actinomyces nasicola* sp. nov.

The bacterial isolate R2014^T was referred to the Anaerobe Reference Unit, PHLS, University Hospital of Wales, for identification. The strain was recovered from an 81-year-old male patient admitted for routine nasal polypectomy. Pus was noted in one antrum and was aspirated. This showed numerous polymorphs and branching Gram-positive, diphtheroid-shaped organisms from which strain R2014^T was isolated. The isolate was characterized biochemically by using both conventional tests (Phillips, 1976) and the commercially available API Rapid ID 32A, API Rapid ID 32Strep and API ZYM systems according to the manufacturer's instructions (API bioMérieux). Long-chain cellular fatty acids were analysed as described by Kämpfer & Kroppenstedt (1996). Quinones were analysed as described by Collins (1994). The G + C content (mol%) of the DNA was determined by HPLC according to Mesbah *et al.* (1989). Amplified 16S rDNA restriction analyses were performed using *Hae*III and *Hpa*II, as described previously (Hall *et al.*, 1999). The 16S rRNA gene of the isolate was amplified by a PCR and sequenced directly using a *Taq* Dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the novel isolate were determined by performing GenBank/EMBL/DBJ database searches. These sequences and those of other known related strains were retrieved from GenBank/

The GenBank accession number for the 16S rRNA sequence of CCUG 46092^T is AJ508455.

EMBL/DDBJ and aligned with the newly determined sequence using the program DNATools (Rasmussen, 1995). The resulting multiple sequence alignment was corrected manually using the program GeneDoc (Nicholas *et al.*, 1997) and a distance matrix was calculated using the program DNADIST (using the Kimura-2 correction parameter) (Felsenstein, 1989). A phylogenetic tree was constructed, according to the neighbour-joining method, with the program NEIGHBOR (Felsenstein, 1989). The stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989). Maximum-parsimony analysis was also performed (Felsenstein, 1989).

The unidentified micro-organism from antral washout consisted of Gram-positive, short, diphtheroid-shaped rods; some branching and coccoid forms were observed. It was non-acid-fast and non-spore-forming. The micro-organism was facultatively anaerobic, but grew better under anaerobic conditions. Acid was formed from fructose and cellobiose but not from amygdalin, arabinose, glucose, lactose, mannitol, mannose, raffinose, ribose, salicin, sucrose, trehalose or xylose in conventional tests. The end-products of glucose metabolism were acetic, lactic and succinic acids. The organism failed to hydrolyse aesculin, gelatin and starch, did not produce indole and was lecithinase-, lipase- and urease-negative. In tests using the API Rapid ID 32A kit, the isolate displayed activity for alanine arylamidase, arginine arylamidase, β -galactosidase, α -glucosidase, β -glucosidase, glycine arylamidase, histidine arylamidase, proline arylamidase, leucine arylamidase, *N*-acetyl- β -glucosaminidase, phenylalanine arylamidase, serine arylamidase and tyrosine arylamidase. Activity for arginine dihydrolase, pyroglutamic acid arylamidase and glutamyl glutamic acid arylamidase was either weak or absent. All other tests in the API Rapid ID 32A kit gave negative results. With the API Rapid ID 32Strep system, the isolate showed activity for alanine-phenylalanine-proline arylamidase, β -galactosidase, β -glucosidase and *N*-acetyl- β -glucosaminidase but gave negative reactions for all of the other tests. With the API ZYM test kit, activity for β -galactosidase, α -glucosidase, leucine arylamidase, *N*-acetyl- β -glucosaminidase and valine arylamidase was detected but all other enzyme tests were negative.

The long-chain cellular fatty acids of the organism consisted of a complex mixture of straight-chain saturated, monounsaturated, iso-methyl-branched and anteiso-methyl-branched types, with anteiso-C15 (13%), C16:0 (29%), C18:0 (11%) and C18:1 ω 9c (26%) as the major acids. Other acids were present in relatively small amounts [iso-C14 (1.5%), C14:0 (7.5%), iso-C15 (3%), C15:0 (1%), iso-C16:0 (2%), C16:1 (0.5%), iso-C17 (1.5%), anteiso-C17 (1.5%) and C17:0 (1%)]. The organism contained menaquinones as the sole respiratory lipoquinones, with MK-9 (90%) as the major component, together with small amounts of MK-10 (10%). The cellular morphology and biochemical

reactions of the micro-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. To clarify the genetic relatedness of the unidentified micro-organism to *Actinomyces* species, amplified 16S rDNA restriction analyses were performed. The unknown micro-organism produced a unique 16S rDNA restriction pattern with *Hae*III and *Hpa*II (profile 072/058) that was distinct from the profiles derived from analysis of over 400 *Actinomyces* strains (Hall *et al.*, 2001). To investigate the phylogenetic relationships of the unknown micro-organism, its almost complete 16S rRNA gene sequence (>1400 nt) was determined. Sequence database searches confirmed that the unknown isolate was most closely related to species of the genus *Actinomyces*. The highest sequence relatedness was shown to *Actinomyces hordeovulneris* CIP 103149^T (93.4%), *Actinomyces canis* CCUG 41706^T (91.8%) and *Actinomyces marimammalium* CCUG 41710^T (91.4%), with other *Actinomyces* species displaying lower levels of relatedness (<91%; data not shown). Neighbour-joining treeing analysis further demonstrated the distinctiveness of the clinical isolate, with the organism forming a distinct subline, clustering with *A. hordeovulneris* CIP 103149^T (Fig. 1). Parsimony analysis confirmed the affinity between the unidentified bacterium and *A. hordeovulneris*.

It is evident from the findings presented that the unidentified facultatively anaerobic, catalase-negative, rod-shaped micro-organism represents another hitherto unknown *Actinomyces* species from clinical sources. Comparative 16S rRNA gene sequencing shows that the unknown isolate forms a distinct subline within the genus *Actinomyces* and displays a specific affinity with *A. hordeovulneris* CIP 103149^T, a species associated with canine actinomycosis (Buchanan *et al.*, 1984). Bootstrap resampling showed that the clustering of the unknown bacterium with *A. hordeovulneris* was statistically significant (91% bootstrap value based on 500 tree replications). However, a 16S rDNA sequence divergence of approximately 6.6% between the unidentified isolate and the type strain of *A. hordeovulneris* showed that this association was not particularly close. Although there is no precise correlation between percentage 16S rDNA sequence divergence values and species delineation, it is now generally accepted that micro-organisms displaying values of 3% or more do not belong to the same species (Stackebrandt & Goebel, 1994). The observed >6% sequence divergence between the unidentified clinical isolate and *A. hordeovulneris* therefore demonstrates clearly that these micro-organisms represent quite different species. The next nearest phylogenetic relative of the unknown clinical bacterium corresponds to *A. marimammalium*. However, sequence divergence considerations and treeing analysis indicate that this association is relatively loose. Support for the separateness of the unknown bacterium also comes from phenotypic evidence. In particular, the biochemical profile of the human clinical isolate serves to distinguish it from all currently recognized *Actinomyces* species. The unidentified isolate can be readily

Fig. 1. Tree showing the phylogenetic relationships of *Actinomyces nasicola* sp. nov. CCUG 46092^T and its nearest relatives in the genus *Actinomyces*. The tree, constructed using the neighbour-joining method, was based on a comparison of approx. 1327 nucleotides. Bootstrap values, expressed as percentages of 500 replications, are given at branching points. Bar, 1 %.

distinguished from its closest relative, *A. hordeovulneris*, by several biochemical traits. In particular, the human clinical isolate ferments a much narrower range of carbohydrates than *A. hordeovulneris*. In addition, the dog pathogen *A. hordeovulneris* can be differentiated from the human clinical isolate by its positive catalase, α -galactosidase and glycine-tryptophan arylamidase reactions, the unknown clinical bacterium showing the opposite response. Tests (in the commercially available API Rapid ID 32Strep system) that are useful in distinguishing the novel bacterium from *A. hordeovulneris* and its next nearest phylogenetic relative, *A. marimammalium*, are shown in Table 1.

On the basis of the distinct phenotypic characteristics of the unidentified rod-shaped bacterium and the molecular chemical and molecular genetic evidence, we consider that this micro-organism warrants classification as a novel

species of the genus *Actinomyces*, for which the name *Actinomyces nasicola* sp. nov. is proposed. Although only a single strain of *A. nasicola* is currently known, we consider that the formal description of this species, together with biochemical and chemical criteria to assist with its identification, will facilitate its recognition in clinical laboratories, thereby permitting the recovery of additional strains of this species and an evaluation of its distribution, clinical prevalence and possible significance.

Description of *Actinomyces nasicola* sp. nov.

Actinomyces nasicola (na.si'co.la. L. masc. n. *nasus* nose; L. masc. suffix *-cola* inhabitant of; N.L. masc. n. *nasicola* inhabitant of the nose, referring to the place of isolation of the type strain).

Cells are Gram-positive, short, diphtheroid-shaped rods; some branching and coccoid forms occur. Non-acid-fast and non-spore-forming. After 48 h anaerobic incubation on fastidious anaerobe agar, colonies are pinpoint, white or grey, opaque, shiny, entire and convex. Facultatively anaerobic; grows in air but grows better under anaerobic conditions. Catalase-negative. Acid is formed from fructose and cellobiose but not from amygdalin, arabinose, glucose, lactose, mannitol, mannose, raffinose, ribose, salicin, sucrose, trehalose or xylose in conventional tests. Acetic, lactic and succinic acids are the end-products of glucose metabolism. Aesculin, gelatin and starch are not hydrolysed. Lecithinase, lipase and urease are not produced. With API systems, acid is not produced from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, maltose, mannitol, mannose, melibiose, melezitose, methyl β -D-glucopyranoside, pullulan, raffinose, ribose, sorbitol, sucrose, tagatose, trehalose or D-xylose. Hippurate is not hydrolysed, indole is not produced and nitrate is not reduced. Activity is detected for alanine arylamidase, alanine-phenylalanine-proline arylamidase, arginine arylamidase, β -galactosidase, α -glucosidase, glycine arylamidase, histidine arylamidase, proline arylamidase, leucine

Table 1. Tests that are useful in distinguishing *A. nasicola* sp. nov. from its closest phylogenetic relatives using the API Rapid ID 32Strep system

Strains: 1, *A. hordeovulneris* CCUG 32937^T, CCUG 34476 and CCUG 34478; 2, *A. marimammalium* CCUG 41710^T and CCUG 42833; 3, *A. nasicola* sp. nov. CCUG 46092^T. +, Positive; –, negative; V, variable.

Test	1	2	3
Acid from:			
Glycogen	V	V	–
Lactose	+	V	–
Pullulan	+	–	–
Sucrose	+	–	–
Production of:			
α -Galactosidase	+	–	–
β -Glucosidase	+	–	+
Glycine-tryptophan arylamidase	+	+	–

arylamidase, *N*-acetyl- β -glucosaminidase, phenylalanine arylamidase, serine arylamidase, valine arylamidase or tyrosine arylamidase. Activity may or may not be detected for arginine dihydrolase, glutamyl glutamic acid arylamidase, pyroglutamic acid arylamidase and β -glucosidase. No activity is detected for α -arabinosidase, acid phosphatase, alkaline phosphatase, cysteine arylamidase, esterase C₄, ester lipase C₈, α -fucosidase, α -galactosidase, β -galactosidase-6-phosphate, glycine-tryptophan arylamidase, glutamic acid decarboxylase, β -glucuronidase, lipase C₁₄, α -mannosidase, β -mannosidase, phosphoamidase, pyrrolidonyl arylamidase, chymotrypsin, trypsin or urease. Voges-Proskauer-negative. The major respiratory quinone is MK-9. The long-chain cellular fatty acids are of the straight-chain saturated, monounsaturated, iso-methyl-branched and anteiso-methyl-branched types, with anteiso-C15, C16:0, C18:0 and C18:1 ω 9c as the major components. The G+C content of the DNA of the type strain is 66.5 mol%.

The type strain, R2014^T (=CCUG 46092^T = CIP 107668^T), was isolated from antrum aspirate. Habitat unknown.

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