Actinobaculum urinale sp. nov., from human urine

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A hitherto undescribed *Actinomyces*-like bacterium was isolated from human urine. Based on its biochemical characteristics, the unidentified bacterium did not correspond to any currently described *Actinomyces* species or related taxa. Comparative 16S rRNA gene sequencing showed that the unknown bacterium exhibits a specific phylogenetic association with the genus *Actinobaculum*, but a sequence divergence of > 5 % from the two currently recognized members of this genus, *Actinobaculum schaalii* and *Actinobaculum suis*, demonstrates that it represents a distinct species. Based on both phenotypic and 16S rRNA gene sequence considerations, it is proposed that the unknown bacterium from urine should be classified as a novel species, *Actinobaculum urinale* sp. nov. The type strain of *Actinobaculum urinale* is CCUG 46093^T (= CIP 107424^T).

The genus *Actinobaculum* Lawson *et al.* 1997 forms a distinct subline, related to but distinct from the genera *Actinomyces*, *Arcanobacterium* and *Mobiluncus*. The genus *Actinobaculum* was proposed to accommodate organisms previously designated as *Actinomyces suis*, and also some *Actinomyces*-like organisms originating from human sources (Lawson *et al.*, 1997). Currently, two species, *Actinobaculum suis* and *Actinobaculum schaalii*, are recognized in this genus; *Actinobaculum suis*, the type species of the genus, is an established pathogen of pigs and has had a chequered taxonomic history, having been assigned to a variety of genera including *Corynebacterium*, *Eubacterium* and *Actinomyces* (Wegienek & Reddy, 1982). The second species within the genus, *Actinobaculum schaalii*, has been recovered from human clinical specimens such as blood and urine, but its pathogenic potential remains unknown. In recent years, there has been increased interest in *Actinomyces* and related organisms as possible opportunistic human pathogens, and a great variety of novel species have been described from clinical specimens, where they are associated with disease and/or occur as mucosal contaminants (e.g. Collins *et al.*, 2000; Hall *et al.*, 1999, 2001; Nikolaitchouk *et al.*, 2000; Schaal, 1998; Wüst *et al.*, 1995). During the course of an ongoing study of clinical isolates of *Actinomyces* and related taxa from human sources (Hall *et al.*, 1999, 2001), we have characterized a hitherto unknown *Actinobaculum*-like organism from urine. Based on both phenotypic and phylogenetic evidence, we propose

a novel species of the genus *Actinobaculum*, with the name *Actinobaculum urinale* sp. nov.

Strain R9242^T was isolated in 1995 in Worthing, West Sussex, UK, from an anaerobic culture of urine from a human female with pyuria, after repeated urine specimens from this patient yielded no bacterial growth under aerobic conditions. The isolate grew poorly in air plus 5 % CO₂, but failed to grow aerobically and was considered to be clinically significant. Strain R9242^T has been deposited in the Culture Collection of the University of Göteborg under the accession number CCUG 46093^T. The strain was cultured on Columbia agar (Difco) supplemented with 5 % horse blood at 37 °C, under anaerobic conditions. The strain was characterized biochemically by using the API Rapid ID 32Strep, API Rapid ID 32A, API Coryne and API ZYM systems, according to the manufacturer's instructions (bioMérieux). Volatile and non-volatile end products of glucose metabolism were detected by GLC (Holdeman *et al.*, 1977). Long-chain cellular fatty acids were analysed as described by Kämpfer & Kroppenstedt (1996). The 16S rRNA gene of the isolate was amplified by PCR and sequenced directly, using a Taq DyeDeoxy Terminator Cycle Sequencing kit and a model 373A automatic DNA sequencer (both from Applied Biosystems). The closest known relatives of the new isolate were determined by performing database searches. These sequences, and those of other known related strains, were retrieved from GenBank and aligned with the newly determined sequence using the program DNATools (Rasmussen, 1995). The resulting multiple sequence alignment was corrected manually, and a distance matrix was calculated with the programs PRETTY and DNADIST, using Kimura's

The GenBank accession number for the 16S rRNA gene sequence of CCUG 46093^T is AJ439453.

two-parameter correction (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).

The new isolate consisted of Gram-positive, non-spore-forming, non-acid-fast, straight to slightly curved rods. After 48 h anaerobic incubation on fastidious anaerobe agar (LAB M) with 5 % horse blood, colonies were <1 mm in diameter, convex, smooth, entire-edged, grey or white and weakly β -haemolytic. On primary culture, the isolate grew well under anaerobic conditions, poorly in air plus 5 % CO₂ and not at all in air. After repeated subcultures, weak growth under aerobic conditions was achieved. In air and in air plus CO₂, colonies were smaller but had a similar morphology to that seen under anaerobic conditions. The organism was catalase-negative and produced L-lactic acid as the major product of glucose metabolism, together with minor amounts of acetic acid. Using the API Rapid ID 32Strep system, the isolate produced acid from ribose but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, mannitol, maltose, melibiose, melezitose, methyl β -D-glucopyranoside, pullulan, raffinose, sorbitol, sucrose, tagatose or trehalose. The organism hydrolysed hippurate and was β -glucuronidase-positive. All other tests in the API Rapid ID 32Strep system were negative. Using the API Coryne system, the isolate produced acid from glucose, ribose, maltose and sucrose. Acid formation from the latter two substrates was at variance with the results from the API Rapid ID 32Strep system. With the API Coryne system, the organism tested positive for β -glucuronidase and urease production; all other enzyme tests were negative using this test kit. The code generated using the API Coryne system corresponded to 0201321. Using the API Rapid ID 32A system, the isolate gave positive reactions for arginine

arylamidase, β -glucuronidase, proline aroylamidase and urease; no other enzyme activities were detected with this system. Employing the API ZYM system, only β -glucuronidase activity was positive. On Christensen's urea agar, a rapid positive reaction was observed.

The long-chain cellular fatty acid composition of the isolate was examined and found to consist of C_{10:0} (1 %), C_{12:0} (2 %), C_{14:0} (19 %), C_{16:0} (30 %), C_{16:1} (7 %), C_{18:0} (7 %) and C_{18:1 ω 9c} (34 %). This fatty acid composition is consistent with that found in species of the genera *Actinomyces*, *Arcanobacterium* and *Actinobaculum*. Based on its cellular morphology and general biochemical reactions, the unidentified organism did not correspond to any recognized species within the genus *Actinomyces* or related genera (Schaal, 1986, 1998). To establish the phylogenetic affinities of the organism, its almost-complete 16S rRNA gene sequence was determined. Sequence database searches showed that the organism was related to the high-G+C, Gram-positive *Actinobacteria*. The organism had highest sequence similarity to members of the genera *Actinobaculum* and *Arcanobacterium*, with species of the genera *Actinomyces* and *Mobiluncus* being more distantly related (data not shown). Neighbour-joining treeing analysis showed that the novel isolate possessed a significant phylogenetic affinity with the genus *Actinobaculum* (Fig. 1). The clustering of the isolate with the *Actinobaculum* group was supported by a bootstrap resampling value of 90 %. Sequence similarities between the novel isolate and *Actinobaculum schaalii* and *Actinobaculum suis* were respectively 93.3 and 92.0 %.

It is apparent from comparative 16S rRNA gene sequencing that the isolate recovered from human urine represents a hitherto unknown *Actinobaculum* species. Only two members of this genus, *Actinobaculum schaalii* and *Actinobaculum suis*, have been described at the time of writing (Lawson *et al.*, 1997). It is evident from the treeing analysis that the bacterium represents a novel subline,

Fig. 1. Unrooted neighbour-joining tree showing the phylogenetic relationships of *Actinobaculum urinale* sp. nov. The tree was based on a comparison of approximately 1327 nt. Bootstrap values, expressed as percentages of 500 replications, are given at branching points. Bar, 1 % sequence divergence.

branching at the base of the *Actinobaculum* cluster. Sequence divergence values of > 5 % between the unidentified isolate and *Actinobaculum schaalii* and *Actinobaculum suis*, however, show that the phylogenetic affinity is not particularly close and that the novel isolate merits separate species status. The separateness of the novel isolate is also very evident from biochemical criteria. Biochemically, the organism is quite different from *Actinobaculum schaalii* and *Actinobaculum suis*, and can be readily distinguished from all other *Actinomyces*-like species. Thus, based on the results of the reported taxonomic study, we propose that the novel isolate from urine should be classified in the genus *Actinobaculum*, as *Actinobaculum urinale* sp. nov. Tests that are useful for distinguishing *Actinobaculum urinale* from other species of the same genus are shown in Table 1. Although only a single strain of *Actinobaculum urinale* is currently known, we consider that the formal description of this species, together with biochemical criteria to aid its identification, will facilitate its recognition in the future 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 *Actinobaculum urinale* sp. nov.

Actinobaculum urinale (u.ri.na'le. N.L. neut. adj. *urinale* pertaining to urine).

Cells are straight to slightly curved Gram-positive rods. Branching is not observed. Cells are non-acid-fast and non-spore-forming. After 48 h anaerobic incubation on fastidious anaerobe agar with 5 % horse blood, colonies are < 1 mm in diameter, convex, smooth, entire-edged, grey or white and weakly β -haemolytic. Facultatively anaerobic and catalase-negative. Lactic acid is the major end product of glucose metabolism, together with minor amounts of acetic acid. Using the commercially available API systems, acid is produced from glucose and ribose; acid production from maltose and sucrose is variable and dependent on the test system used. Acid is not formed from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, mannitol, mannose, melibiose, melezitose, methyl β -D-glucopyranoside, pullulan, raffinose, sorbitol, tagatose, trehalose or D-xylose. Hippurate is hydrolysed but gelatin and aesculin are not. Arginine arylamidase, β -glucuronidase and proline arylamidase are produced and urease may or may not be detected. Alanine-phenylalanine-proline arylamidase, arginine dihydrolase, acid phosphatase, alkaline phosphatase, alanine arylamidase, arginine arylamidase, α -arabinosidase, chymotrypsin, trypsin, cysteine arylamidase, esterase C-4, ester lipase C-8, α -fucosidase, α -galactosidase, β -galactosidase, β -galactosidase-6-phosphate, α -glucosidase, β -glucosidase, glycyl tryptophan arylamidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase,

Table 1. Commercial tests that are useful for distinguishing *Actinobaculum urinale* sp. nov. from *Actinobaculum schaalii* and *Actinobaculum suis*

A. schaalii strains tested were CCUG 27420^T, CCUG 19698, CCUG 32296, CCUG 29359 and CCUG 36567. +, Positive; –, negative; v, variable.

Test	<i>A. urinale</i> CCUG 46093 ^T	<i>A. schaalii</i> (n=5)	<i>A. suis</i> CCUG 19206 ^T
API Rapid ID 32Strep system			
Acid from:			
L-Arabinose	–	v	–
Maltose	–	+	–
Sucrose	–	v	–
Production of:			
Alanine-phenylalanine-proline arylamidase	–	+	+
Alkaline phosphatase	–	–	+
β -Glucuronidase	+	–	+
Pyroglutamic acid arylamidase	–	+	+
API Rapid ID 32A system			
Production of:			
Alanine arylamidase	–	+	+
Alkaline phosphatase	–	–	+
Glycine arylamidase	–	+	+
α -Glucosidase	–	+	+
β -Glucuronidase	+	–	+
Leucine-glycine arylamidase	–	+	+
Pyroglutamic acid arylamidase	–	+	+
Tyrosine arylamidase	–	+	+
Urease	+	–	+

leucyl glycine arylamidase, lipase C-14, α -mannosidase, β -mannosidase, *N*-acetyl- β -glucosaminidase, phosphoamidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, pyrazinamidase, serine arylamidase, tyrosine arylamidase and valine arylamidase are not detected. Acetoin is not produced. Indole-negative. Nitrate is not reduced to nitrite. The cellular fatty acids are of the straight-chain saturated and monounsaturated types, with C_{14:0}, C_{16:0}, C_{16:1}, C_{18:0} and C_{18:1} ω 9c predominating.

The type strain, CCUG 46093^T (=CIP 107424^T), was isolated from human urine. Habitat is not known.

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