NOTE

Characterization of *Actinomyces* isolates from samples from the human urogenital tract: description of *Actinomyces urogenitalis* sp. nov.

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Three strains of a previously undescribed *Actinomyces*-like bacterium were isolated from human clinical sources (urine, urethra and vaginal secretion). Biochemical testing and PAGE analysis of whole-cell proteins indicated that the strains were phenotypically homogeneous and distinct from previously described *Actinomyces* and *Arcanobacterium* species. Comparative 16S rRNA gene sequencing studies showed the bacterium to be a hitherto unknown subspecies within a group of *Actinomyces* species which includes *Actinomyces bovis*, the type species of the genus. Based on phylogenetic and phenotypic evidence it is proposed that the unknown bacterium from humans be classified as *Actinomyces urogenitalis* sp. nov. The type strain of *Actinomyces urogenitalis* is CCUG 38702^T (= CIP 106421^T).

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The genus *Actinomyces* embraces a phenotypically heterogeneous group of anaerobic and facultatively anaerobic, asporogenous, Gram-positive, non-acid-fast, filamentous or diphtheroidal rod-shaped organisms (Schaal, 1986). The taxonomy of the genus *Actinomyces* has long been recognized as being unsatisfactory (Schaal, 1986). During the past few years, *Actinomyces* taxonomy has undergone much improvement primarily driven by 16S rRNA gene sequence analysis (e.g. Pascual *et al.*, 1997a, b; Lawson *et al.*, 1997). It is now clear that the genus *Actinomyces* as presently defined is phylogenetically very heterogeneous and in fact encompasses several species and groups of species worthy of separate generic status. As a result, the dissection of the *Actinomyces* genus has commenced with the creation of the genus *Actinobaculum* (Lawson *et al.*, 1997). A second major taxonomic development has been a considerable expansion in the number of described *Actinomyces* and related species [e.g. *Actinomyces europaeus* (Funke *et al.*, 1997); *Actinomyces graevenitzii* (Pascual *et al.*,

1997b); *Actinomyces neuui* subsp. *anitratus* and *neuui* (Funke *et al.*, 1994); *Actinomyces hyovaginalis* (Collins *et al.*, 1993); *Actinomyces radingae* (Wüst *et al.*, 1995); *Actinomyces turicensis* (Wüst *et al.*, 1995); *Actinobaculum schaalii* (Lawson *et al.*, 1997); *Arcanobacterium bernardiae* (Funke *et al.*, 1995); *Arcanobacterium phocae* (Pascual *et al.*, 1997a)]. The recognition of these new *Actinomyces* and related taxa has in the main been due to the use of phenotypic and genotypic information in concert (polyphasic taxonomy). Most of the newly described organisms have been from human sources stimulated by an increased interest in these organisms as opportunistic pathogens. In this article, we report the results of a polyphasic taxonomic study on three strains of an unusual *Actinomyces*-like bacterium from human clinical specimens. Based on the presented findings, yet another new species of the genus *Actinomyces*, *Actinomyces urogenitalis*, is described.

Three human clinical isolates (CCUG 28744, CCUG 38702^T and CCUG 42029) were referred to the Culture Collection of the University of Göteborg, Sweden, for identification. Strain CCUG 28744 was cultured from urine (10⁷ c.f.u. ml⁻¹) of a 70-year-old woman whereas strain CCUG 38702^T was isolated from vaginal se-

Abbreviation: CCUG, Culture Collection of the University of Göteborg.
The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 38702^T is AJ243791.

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

cretion of a 33-year-old woman who had abnormal discharges, possibly due to the use of an intrauterine device for 7 years. Strain CCUG 42029 was recovered from human urethra (44-year-old patient). The unidentified *Actinomyces*-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). For densitometric analysis, normalization and interpret-

ation of protein patterns, the GCW 4.0 software package (Applied Maths, Kortrijk, Belgium) was used. The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient converted for convenience to a percentage similarity. The G + C content of DNA was determined by thermal denaturation as described by Farrow *et al.* (1983). The 16S rRNA genes of representative isolates were amplified by PCR and directly sequenced using a *Taq* DyeDeoxy Terminator Cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolates were determined by

performing database searches. These sequences and those of other known related strains were retrieved from the GenBank or Ribosomal Database Project (RDP) Libraries and aligned with the newly determined sequences using the program PILEUP (Devereux *et al.*, 1984). The resulting multiple sequence alignment was corrected manually and a distance matrix was calculated using the programs PRETTY and 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). Parsimony analysis was also performed using the same package (Felsenstein, 1989). The 16S rRNA gene sequence of strain CCUG 38702^T has been deposited in GenBank under accession number AJ243791.

The three isolates were Gram-positive straight to slightly curved rods which were non-acid-fast and non-spore-forming. The strains were catalase-negative and phenotypically closely resembled each other, producing acid from glucose, D-arabitol, maltose, melibiose, melezitose, lactose, D-raffinose, sucrose, trehalose and D-xylose. None of the isolates produced acid from cyclodextrin, glycogen, pullulan, sorbitol or tagatose. Alanine phenylalanine proline arylamidase, α -glucosidase, β -glucosidase, α -galactosidase, β -galactosidase, β -galacturonidase, *N*-acetyl- β -glucosamine, pyrrolidonyl arylamidase, pyroglutamic acid arylamidase, valine arylamidase and leucine arylamidase were produced by the isolates but tests for arginine dihydrolase, acid phosphatase, chymotrypsin, esterase C-4, ester lipase C8, α -fucosidase, β -glucuronidase, glycyl tryptophane arylamidase, β -mannosidase, lipase C14, pyrazinamidase, trypsin and urease were negative. Variable reactions were observed for alkaline phosphatase, α -mannosidase and cystine arylamidase. All of the isolates hydrolysed aesculin but not gelatin or hippurate. The cellular morphology and biochemical reactions of the isolates were consistent with their assignment to the genus *Actinomyces*. To assess the phenotypic resemblance of the three isolates to each other and to reference *Actinomyces* species, a comparative analysis of whole-cell protein profiles by SDS-PAGE was performed. The three isolates clustered together and formed a distinct group with a within-group correlation level of 85% or more. *Actinomyces slackii*, *Actinomyces turicensis* and *Actinobaculum schaalii* were the nearest species to the unknown isolates, joining the cluster at a correlation level of about 60% (Fig. 1). The PAGE results confirmed that the three unidentified strains represent a phenotypically homogeneous group of organisms and that they are distinct from all *Actinomyces* species and close relatives described to date. To ascertain the phylogenetic relationships of the clinical isolates, their 16S rRNA genes were sequenced and subjected to a comparative analysis. The almost complete gene

sequences (>1500 nucleotides) of the three strains were determined and pairwise analysis showed these to be almost identical (99.8–100% similarity). Sequence database searches confirmed the unknown bacterium was most closely related to species of the genus *Actinomyces* (results not shown). Highest sequence relatedness was shown with *Actinomyces bovis*, *Actinomyces bowdenii*, *Actinomyces naeslundii*, *Actinomyces viscosus* and *Actinomyces slackii*. The results of neighbour-joining analysis are shown in Fig. 2 and confirmed the association of the unknown clinical bacterium (as exemplified by strain CCUG 38702^T) with *Actinomyces bovis* and its near relatives.

Earlier 16S rRNA sequencing studies (Pascual *et al.*, 1997a; Lawson *et al.*, 1997) have shown that the genus *Actinomyces* is not monophyletic and consists of several lineages worthy of separate generic status. It is clear from the present 16S rRNA study that the novel bacterium reported here forms a distinct subline within a cluster of species which includes the type species *Actinomyces bovis* and, therefore, can be regarded as an authentic *Actinomyces* species. In terms of evolutionary distances, the unknown organism from clinical sources is approximately equidistant from *Actinomyces bovis* and *Actinomyces slackii* (its nearest phylogenetic relatives) and sequence divergence values of 3.3 and 3.1% with these species, respectively, are indicative of a phylogenetically distinct species. In the treeing analysis, the association of the unidentified clinical bacterium with *Actinomyces bovis* was recovered in only 40% of bootstrap replicates. The observed low bootstrap resampling value probably reflects the approximate equidistance the bacterium shows with *Actinomyces bovis* and *Actinomyces slackii*. Although there is no precise correlation between percentage 16S rRNA divergence values and species delineation, it is generally recognized that organisms displaying values close to 3% do not belong to the same species (Stackebrandt & Goebel, 1994). The observed >3% sequence divergence between the unknown rod-shaped organism and *Actinomyces bovis* and *Actinomyces slackii* is consistent with this guideline. It is pertinent to note that many described genomically distinct species within the *Actinobacteria* display much smaller levels of sequence divergence (i.e. less than 1 or 2%). The observed >3% divergence between the unknown organism and other members of the *Actinomyces bovis* cluster of species is also very much greater than that which may be expected between different strains of the same species. Multiple strains of several *Actinomyces* species (and species of related genera such as *Arcanobacterium* and *Actinobaculum*) have been examined by partial and/or near complete 16S rRNA gene sequencing and levels of divergence of 0.5% or less have invariably been found. Hence the 0.2% divergence observed between the three unknown clinical isolates and >3% divergence shown with respect to *Actinomyces bovis* and *Actinomyces slackii* is considered very significant. In addition to the aforementioned genetic findings, the unknown isolates are

Fig. 2. Unrooted tree showing the phylogenetic relationships of *Actinomyces urogenitalis* sp. nov. and some other high G+C content Gram-positive bacteria. 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. Bar, 1%.

phenotypically very distinct from *Actinomyces bovis*, *Actinomyces slackii* and related species. The unknown rod-shaped bacterium differs from other species of the *Actinomyces bovis* cluster of organisms in numerous independent physiological and biochemical traits (see Table 1). Strong support for the separateness of the unknown clinical bacterium also comes from PAGE whole-cell protein profiling (Fig. 1). It is now firmly established that this molecular chemical approach is extremely reliable for comparing closely related strains and shows excellent correlation with DNA–DNA pairing (Vandamme *et al.*, 1996). The PAGE protein profiling results shown in Fig. 1 unequivocally demonstrate that the unknown isolates represent a separate species from *Actinomyces bovis*, *Actinomyces slackii* and other close relatives. Therefore, based on the

distinct phenotypic characteristics of the unknown rod-shaped bacterium, and the use of molecular chemical and molecular genetic evidence in concert, we are of the firm view that it merits classification as a new species of the genus *Actinomyces*, for which the name *Actinomyces urogenitalis* is proposed. Tests which are useful in differentiating *Actinomyces urogenitalis* from its nearest relatives are shown in Table 1. The three isolates of *Actinomyces urogenitalis* were recovered from the human urogenital tract. Although the species is possibly a commensal of the human vagina, it is not known if the organism is a cause of urogenital disease. The formal description of the new species will, however, facilitate its recognition in the clinical laboratory, thereby permitting a future evaluation of any possible clinical significance.

Table 1. Characteristics useful in differentiating *Actinomyces urogenitalis* sp. nov. from its closest phylogenetic relatives

MLZ, melezitose; D-AR, D-arabitol; D-XY, D-xylose; APPA, alanine phenylalanine proline arylamidase; PYRG, pyroglutamic acid arylamidase; α -GLU, α -glucosidase; β -NAG, *N*-acetyl- β -glucosaminidase; ADH, arginine dihydrolase; +, positive; –, negative; v, variable.

Species*	Nitrate reduction	Fermentation of:			Production of:				
		MLZ	D-AR	D-XY	APPA	PYRG	α -GLU	β -NAG	ADH
<i>Actinomyces bowdenii</i> (4)	+	+	–	–	+	–	v	–	–
<i>Actinomyces bovis</i> (6)	–	–	–	–	–	v	–	v	–
<i>Actinomyces denticolens</i> (13)	+	v	–	v	v	–	+	–	–
<i>Actinomyces howellii</i> (2)	–	v	–	+	+	–	+	v	+
<i>Actinomyces naeslundii</i> (7)	+	–	–	–	–	–	–	–	–
<i>Actinomyces slackii</i> (4)	+	–	–	+	–	–	+	–	–
<i>Actinomyces viscosus</i> (16)	+	–	–	–	+	–	v	–	–
<i>Actinomyces urogenitalis</i> (3)	+	+	+	+	+	+	+	+	–

* Values in parentheses are the number of strains tested.

Description of *Actinomyces urogenitalis* sp. nov.

Actinomyces urogenitalis (u.ro.ge.ni.ta'lis. M.L. adj. *urogenitalis* belonging to the urogenital tract).

Cells are straight to slightly curved rods which stain Gram-positive, are non-acid-fast and non-motile. Facultatively anaerobic and catalase-negative. Using API systems, acid is produced from D-glucose, D-arabitol (weak), maltose, melibiose, melezitose, lactose, D-raffinose, sucrose, trehalose and D-xylose. Acid is not produced from cyclodextrin, glycogen, pullulan, sorbitol or tagatose. Acid may or may not be produced from L-arabinose, mannitol, methyl- β -D-glucopyranoside and ribose. Aesculin is hydrolysed but gelatin and hippurate are not. Alanine phenylalanine proline arylamidase, α -galactosidase, β -galactosidase, β -galacturonidase, α -glucosidase, β -glucosidase, valine arylamidase, leucine arylamidase, pyrrolidonyl arylamidase, pyroglutamic acid arylamidase and *N*-acetyl- β -glucosaminidase are produced. Arginine dihydrolase, acid phosphatase, chymotrypsin, esterase C-4, ester lipase C8, lipase C14, α -fucosidase, β -glucuronidase, glycyl tryptophane arylamidase, β -mannosidase, pyrazinamidase, trypsin and urease are not produced. Activities for alkaline phosphatase, cystine arylamidase and α -mannosidase may or may not be detected. Acetoin production is variable. Nitrate is reduced to nitrite. Isolated from human clinical sources. Habitat is not known. The type strain is CCUG 38702^T (= CIP 106421^T). The G + C content of the type strain is 61 mol %.

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