An unusual Streptococcus from human urine, Streptococcus urinalis sp. nov.

Matthew D. Collins, Roger A. Hutson, Enevold Falsen, Natalla Nikolaitchouk, Leslye LaClaire and Richard R. Facklam

Author for correspondence: Richard R. Facklam. Tel: +1 404 639 1379. Fax: +1 404 639 3123. e-mail: RRF2@CDC.GOV

Biochemical, molecular chemical and molecular genetic studies were performed on an unknown Gram-positive, catalase-negative, chain-forming coccus isolated from the urine of a patient suffering from cystitis. Comparative 16S rRNA gene sequencing showed that the organism is a member of the ‘pyogenic subgroup’ of the genus Streptococcus and has a close affinity with Streptococcus pyogenes and Streptococcus canis. The unknown coccus was, however, readily distinguished from these species and other streptococci by biochemical tests and electrophoretic analysis of whole-cell proteins. Based on phenotypic and phylogenetic evidence, it is proposed that the unknown bacterium be classified as a new species of the genus Streptococcus, Streptococcus urinalis sp. nov. The type strain of Streptococcus urinalis is CCUG 41590T.

Keywords: 16S rRNA gene, phylogeny, taxonomy, Streptococcus urinalis sp. nov.

The Gram-positive, catalase-negative cocci embrace many agents which are pathogenic to man and other animals (Facklam & Elliot, 1995; Hardie & Whiley, 1991). During the past decade, there has been much change and improvement in the taxonomy and identification of these organisms. In particular, molecular genetic analysis based on 16S rRNA gene sequencing has facilitated new insights into the phylogenetic inter-relationships of the Gram-positive, catalase-negative cocci and provided a powerful means of characterizing new diversity within this important group of bacteria. Indeed, this molecular approach has been primarily responsible for the very considerable increase in the number of newly described Gram-positive, catalase-negative organisms in recent years. Many of these newly delineated organisms represent new members of established genera, such as Enterococcus and Streptococcus, which have long been associated with disease, whereas in other cases, they constitute species of previously unknown genera [e.g. Alloccoccus (Aguirre & Collins, 1992), Dolosigranulum (Aguirre et al., 1993), Globicatella (Collins et al., 1992), Faccklamia (Collins et al., 1993), Helcococcus (Collins et al., 1993) and Ignavigranum (Collins et al., 1999)]. In the past few years, 16S rRNA gene sequencing has been used, in concert with phenotypic tests, to investigate numerous atypical or unknown strains of Gram-positive, catalase-negative cocci from human sources aimed at facilitating their recognition and identification. In the course of this ongoing study, the characteristics of an unknown coccus isolated from human urine are reported. Phylogenetic analysis shows that the organism represents a new subline within the ‘pyogenic subgroup’ of the genus Streptococcus, for which the species name Streptococcus urinalis is proposed.

Strain 2285-97T was sent from the Michigan State Health Department (USA) to the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) for identification. The organism was isolated from the urine of a 55-year-old female patient with cystitis and chronic abdominal pain. The strain has been deposited in the Culture Collection of the University of Göteborg (CCUG), under accession number CCUG 41590T. The unidentified organism was cultured on Columbia agar (Difco) supplemented with 5% sheep blood at 37 °C, in air plus 5% CO₂. The strain was biochemically characterized using the API Rapid ID32 STREP and API ZYM systems according to the manufacturer’s instructions (API bioMérieux). Conventional biochemical and physiological tests were also performed (Facklam & Elliot, 1995). Preparation of cellular protein extracts for PAGE analysis, densitometric analysis, normalization of the protein profiles and
Numerical analysis were performed as described by Pot et al. (1994) using the GELCOMPAR 3.0 software package (Applied Maths, Kortrijk, Belgium). The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient converted for convenience to a percentage similarity value.
The DNA G+C content (mol%) was determined by thermal denaturation as described by Garvie (1978). The 16S rRNA gene(s) of the isolate was 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 isolate were determined by performing database searches. These sequences and those of known related strains were retrieved from the GenBank or Ribosomal Database Project databases and aligned with the newly determined sequence 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-two 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).

The unidentified isolate consisted of Gram-positive, ovoid shaped cells which formed short chains. The organism grew on normal media routinely used for streptococci and was non-haemolytic and non-motile. Employing conventional tests used by CDC (Facklam & Elliot, 1995), the organism did not form gas on MRS broth, was bile/esculin-positive, grew in broth containing 6.5% NaCl and at 45 °C but not at 10 °C. The isolate produced acid in heart infusion base medium containing glucose, lactose, maltose, ribose, sucrose and trehalose, but not from arabinose, glycerol, inulin, melibiose, sorbitol or sorbose. It produced leucine aminopeptidase and pyroglutamic acid arylamidase, melibiose, sorbitol or sorbose. It produced leucine from glucose, lactose, maltose, ribose, sucrose and isoleucine. It produced acid in heart infusion base medium containing trypsin, esterase C4, ester lipase C8, alanine-phenylalanine-proline arylamidase, chymotrypsin, esterase C4, ester lipase C8, x-fucosidase, x-galactosidase, x-galactosidase, x-galacturonidase, β-glucuronidase, glycol-tryptophan arylamidase, lipase C14, x-mannosidase, β-mannosidase, trypsin and valine arylamidase. The organism was Voges–Proskauer-positive. The morphological and biochemical characteristics of the unknown isolate were consistent with its assignment to Streptococcus, although it did not seem to correspond to any of the currently defined species of this genus. To further investigate the phenotypic similarities of the unknown bacterium, PAGE of whole-cell proteins was performed. The results of a numerical analysis of the protein patterns of the unknown coccus and representative strains of currently recognized streptococcal species are shown in Fig. 1. The unidentified isolate clustered with Streptococcus iniae (correlation level approx. 80%), with Streptococcus parauberis as the next nearest relative (joining the cluster at approx. 75%). All other reference species were only remotely related to the unknown coccus. In order to establish the precise phylogenetic relationships of the unknown coccus, its 16S rRNA gene sequence was determined by direct sequencing of in vitro-amplified rRNA gene products. Sequence searches of GenBank and Ribosomal Database Project databases confirmed that the unidentified coccus was phylogenetically most closely related to species of the genus Streptococcus, with enterococci and lactococci being distantly related (less than 90% sequence similarity; data not shown). The unknown coccus also gave a negative reaction with the Enterococcus GenProbe test, thereby confirming the 16S rRNA sequence findings. A tree depicting the phylogenetic position of the unknown bacterium is shown in Fig. 2 and shows that the organism represents a distinct subline within the ‘pyogenic subgroup’ of streptococci, with Streptococcus pyogenes and Streptococcus canis as its nearest phylogenetic relatives. It is clear from both phenotypic and phylogenetic investigations that the unidentified coccus from human urine represents a hitherto unknown Streptococcus species. Phylogenetically, the unknown coccus is clearly a new member of the ‘pyogenic subgroup’ which includes mainly species pathogenic to man and animals (viz. Streptococcus agalactiae, S. canis, Streptococcus dysgalactiae, Streptococcus equi, S. iniae, S. parauberis, S. pyogenes, Streptococcus porcinus and Streptococcus uberis). It is evident from both evolutionary distances and treeing analysis (Fig. 2) that the unknown coccus is approximately equidistant from S. pyogenes and S. canis. The 16S rRNA of the new coccus displayed 34 differences (corresponding to 33 mismatches and 1 unmatched) and 33 differences (corresponding to 32 mismatches and 1 unmatched) to the 16S rRNA of the type strains of S. pyogenes and S. canis, respectively, which is strongly indicative of a phylogenetically distinct species. 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 2.5% sequence divergence between the unknown coccus and S. pyogenes and S. canis is close to the aforementioned guideline. It is pertinent to note that within the genus Streptococcus, several genetically separate species display lower levels of 16S rRNA sequence divergence (e.g. S. canis with S. pyogenes; Streptococcus bovis with Streptococcus macedonicus). The observed 2.5% divergence between the unknown coccus and S.
Unrooted tree based on 16S rRNA showing the phylogenetic position of *Streptococcus urinalis* CCUG 41590T within the genus *Streptococcus*. Numbers on the branches are percentage bootstrap resampling values. Bar, 1% sequence divergence.

*pyogenes*/*S. canis* is also very much greater than that which may be expected between different strains of the same species. For example, in the case of streptococcal species, levels of sequence divergence of 0.5% or less, have been reported for partial and/or near complete 16S rRNAs of strains of the same species [*Streptococcus pharaminalium* (Devriese et al., 1999); *Streptococcus parasanguinis* (Fernandez Garayzabal et al., 1998); *S. parauberis* (Domenech et al., 1996); *Streptococcus hyovaginalis* and *Streptococcus thoraltensis* (Devriese et al., 1997); *Streptococcus infantis* and *Streptococcus peroris* (Kawamura et al., 1998)]. This is also the case for all other Gram-positive, catalase-negative, coccus-shaped taxa for which intra-specific sequence divergence values are available (e.g. *Gemella* spp., *Facklamia* spp.). In addition to the above genetic findings, the unknown isolate is phenotypically quite distinct from *S. pyogenes* and *S. canis*. The unknown coccus can be readily distinguished from *S. pyogenes* and *S. canis* in that it is non-haemolytic and by the absence of group CH antigens, especially group A and G. Both *S. pyogenes* and *S. canis* display strong β-haemolytic activity, and are positive for Lancefield group A and G antigens, respectively (Ruoff, 1991). In addition, the unknown coccus differs from *S. pyogenes* and *S. canis* in numerous other physiological and biochemical traits (see Table 1). Strong support for the separateness of the unidentified coccus 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 hybridizations (Vandamme et al., 1996). The PAGE protein profiling results shown in Fig. 1 unequivocally demonstrate that the unknown isolate is a separate species from *S. pyogenes* and *S. canis*. Therefore, based on the distinct phenotypic characteristics of the unknown coccus, and the use of molecular chemical and molecular genetic evidence in concert, it is firmly believed that the unidentified coccus from human urine represents a hitherto unknown species, for which the name *Streptococcus urinalis* sp. nov. is proposed. It is important to note that currently only a single strain of *Streptococcus urinalis* is known.
**Table 1.** Characteristics useful for differentiating *S. urinalis* from some other pyogenic streptococci

<table>
<thead>
<tr>
<th>Species</th>
<th>Group</th>
<th>Bacitracin*</th>
<th>PYR</th>
<th>CAMP</th>
<th>VP</th>
<th>HIP</th>
<th>Starch</th>
<th>SBL</th>
<th>β-Haemolytic</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. pyogenes</em></td>
<td>A</td>
<td>S</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>B</td>
<td>R</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td><em>S. dysgalactiae</em></td>
<td>subsp. dysgalactiae</td>
<td>C</td>
<td>R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>subsp. equisimilis</td>
<td>C, G, L</td>
<td>R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td><em>S. equi</em></td>
<td>subsp. equi</td>
<td>C</td>
<td>R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>subsp. zooepidemicus</td>
<td>C</td>
<td>R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. canis</em></td>
<td>G</td>
<td>R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>S. parauberis</em></td>
<td>E, none</td>
<td>R</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>S. porcinus</em></td>
<td>E, P, U, V, none†</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>S. iniae</em></td>
<td>None</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td><em>S. phocae</em></td>
<td>C, F, none</td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>S. uberis</em></td>
<td>E, none</td>
<td>R</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td><em>S. urinalis</em>‡</td>
<td>None</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
</tbody>
</table>

* S. Sensitive; R, resistant.
† Some *S. porcinus* strains have group antigens that have not been assigned letters.
‡ The characteristics of *S. urinalis* are based on a single strain.

Although species descriptions based on a single strain are not desirable, the recovery of this organism from a human clinical specimen, together with its distinct taxonomic characteristics, justifies its recognition as a new species. In addition, it is considered that the formal description of *Streptococcus urinalis* will greatly facilitate the isolation of further strains of this species.

**Description of Streptococcus urinalis** sp. nov.

*Streptococcus urinalis* (u.ri.naˈlɪs. M.L. adj. urinalis pertaining to urine).

Cells are Gram-positive, ovoid in shape, occurring singly, in pairs or in short chains. Cells are non-pigmented, non-haemolytic and non-motile. Spores are not produced. Facultatively anaerobic, oxidase-negative and catalase-negative. Growth does not occur at 10 °C. Growth occurs in broth containing 6.5% NaCl. Bile/æsculin-positive. Negative with streptococcal group A, B, C, D, E, F and G antisera. Gas is not produced in MRS broth. Positive for leucine aminopeptidase and pyrrolidonyl arylamidase. Pyruvate is not utilized. Using conventional heart infusion base medium, acid is produced from glucose, lactose, ribose, sucrose, maltose and trehalose. Acid is not produced from arabinose, glycerol, inulin, melibiose, sorbitol or sorbose. Using API systems, acid is produced from maltose, ribose, sucrose and trehalose, but not from β-arabinose, d-arabinot, cyclodextrin, glucogen, lactose, mannotol, melibiose, melezitose, methyl β-D-glucopyranoside, N-acetylglucosamine, pullulan, sorbitol, raffinose or tagatose. It is positive for acid phosphatase, alkaline phosphatase, arginine dihydrolase, α-glucosidase, β-glucosidase, pyrogallol-tamic acid arylamidase and leucine arylamidase, but negative for alanine-phenylalanine-proline arylamidase, chymotrypsin, esterase C4, ester lipase C8, α-fucosidase, α-galactosidase, β-galactosidase, β-galacturonidase, β-glucuronidase, glycy1-tryptophan arylamidase, lipase C14, α-mannosidase, β-mannosidase, trypsin and valine arylamidase. Urea and starch are not hydrolysed. Acetoin is produced. Extracellular polysaccharide is not produced. Acid and clot are formed in litmus milk. Vancomycin-sensitive and bacitracin-resistant. Negative reaction with *Enterococcus* GenProbe test. The G+C content of DNA is 39 mol%. The type strain is CCUG 41590T.

**Acknowledgements**

The excellent SDS-PAGE analysis of Lena Dahl is gratefully acknowledged. We are grateful to Hans Trüper for suggesting the species epithet.

**References**


