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NOTE

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

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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 41590^T.

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 interrelationships 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. *Alloiococcus* (Aguirre & Collins, 1992), *Dolosigranulum* (Aguirre *et al*., 1993), *Globicatella* (Collins *et al*., 1992), *Facklamia* (Collins *et al*., 1997), *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 2285-97^T 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 41590^T. The unidentified organism was cultured on Columbia agar (Difco) supplemented with 5% sheep blood at 37 °C, in air plus 5% CO_2. 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

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 41590^T is AJ131965.

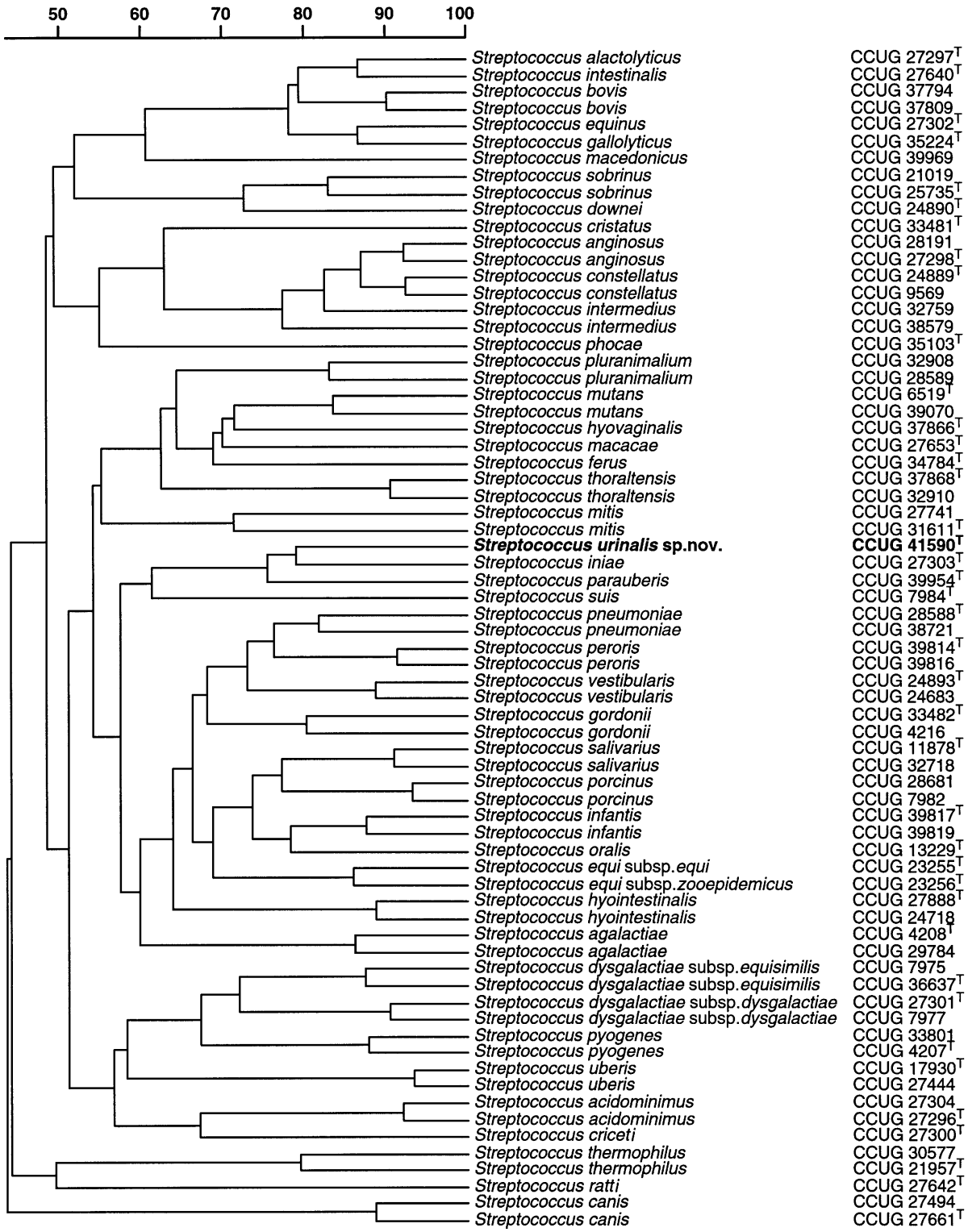

Fig. 1. Dendrogram derived from the unweighted pair group mean linkage of correlation coefficients (expressed as percentage values) between whole-cell protein patterns of *Streptococcus urinalis* CCUG 41590^T and other streptococci.

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/aesculin-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 from 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, hydrolysed aesculin but not urea, hippurate or starch, formed acid and clot in litmus milk, and was vancomycin-sensitive and bacitracin-resistant. Using commercially available API systems, the isolate produced acid from maltose, ribose, sucrose and trehalose, but failed to produce acid from L-arabinose, D-arabitol, cyclodextrin, glycogen, lactose, mannitol, melibiose, melezitose, methyl β-D-glucopyranoside, *N*-acetylglucosamine, pullulan, sorbitol, raffinose or tagatose. It gave positive reactions for acid phosphatase, alkaline phosphatase, arginine dihydrolase, α-glucosidase, β-glucosidase, pyroglutamic acid arylamidase and leucine arylamidase, but was negative for alanine-phenylalanine-proline arylamidase, chymotrypsin, esterase C4, ester lipase C8, α-fucosidase, α-galactosidase, β-galactosidase, β-galacturonidase, β-glucuronidase, glycyl-tryptophan arylamidase, lipase C14, α-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.*

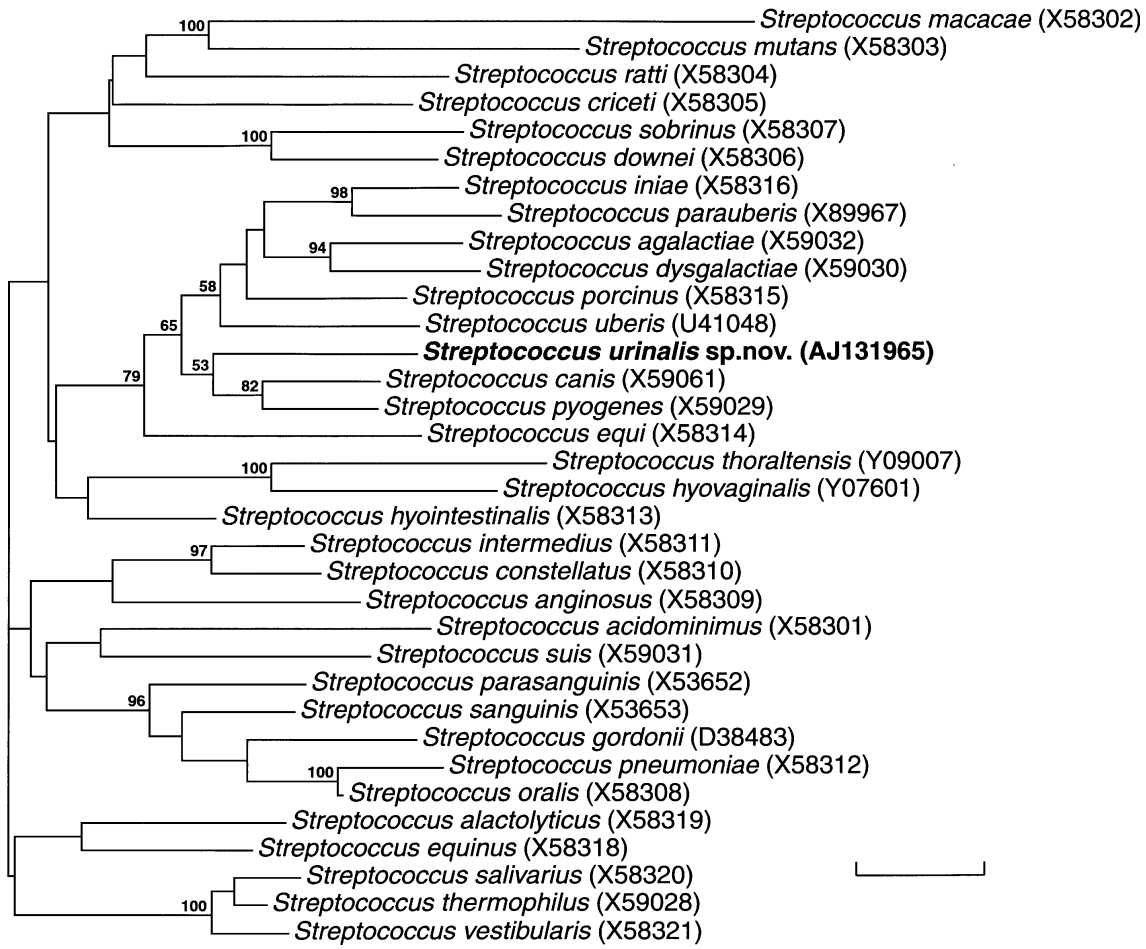

Fig. 2. Unrooted tree based on 16S rRNA showing the phylogenetic position of *Streptococcus urinalis* CCUG 41590^T within the genus *Streptococcus*. Numbers on the branches are percentage bootstrap resampling values. Bar, 1% sequence divergence.

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 pluranimalium* (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

Abbreviations and symbols; Group, Lancefield group antigen; PYR, production of pyrrolidonyl arylamidase; CAMP, Christie, Atkins, Munch-Petersen test; VP, Voges–Proskauer reaction; HIP, hydrolysis of hippurate; Starch, hydrolysis of starch; SBL, acid formation in sorbitol broth. +, Positive; −, negative; V, variable.

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

* 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.na′lis. 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/aesculin-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 L-arabinose, D-arabitol, cyclodextrin, glycogen, lactose, mannitol, 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, pyroglutamic 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, glycyl-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 41590^T.

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