NOTE

***Streptococcus gallinaceus* sp. nov., from chickens**

¹ School of Food Biosciences,
The University of Reading,
Whiteknights, Reading
RG6 6AP, UK

² Culture Collection,
Department of Clinical
Bacteriology, University of
Göteborg, Sweden

³ Department of Veterinary
Microbiology, The Royal
Veterinary and
Agricultural University,
Frederiksberg, Denmark

Matthew D. Collins,¹ Roger A. Hutson,¹ Enevold Falsen,²
Elisabeth Inganäs² and Magne Bisgaard³

Author for correspondence: Matthew D. Collins. Tel: +44 118 935 7226. Fax: +44 118 935 7222.
e-mail: m.d.collins@reading.ac.uk

Three isolates of an unknown Gram-positive, catalase-negative, chain-forming, coccus-shaped organism isolated from an outbreak of septicaemia in a flock of adult broiler parents were characterized by phenotypic and molecular taxonomic methods. Comparative 16S rRNA gene sequencing studies demonstrated that the bacterium represents a new subline within the genus *Streptococcus*, related to, albeit distinct from, *Streptococcus acidominimus*, *Streptococcus ovis*, *Streptococcus suis* and close relatives. The unknown bacterium was readily distinguished from all recognized streptococcal species by biochemical tests. Based on phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium from chickens be classified as *Streptococcus gallinaceus* sp. nov. The type strain is CCUG 42692^T (= CIP 107087^T).

Keywords: *Streptococcus gallinaceus* sp. nov., taxonomy, phylogeny, 16S rRNA

The genus *Streptococcus* embraces a broad range of Gram-positive, catalase-negative, chain-forming, coccus-shaped organisms and, currently, more than 40 species are described. Most streptococcal species are found in association with animal and human sources and many are established and/or opportunistic pathogens. During the past decade, the taxonomy of the genus has been much improved, primarily as a result of the use of improved molecular-based methods such as 16S rRNA gene sequencing and PAGE analysis of whole-cell proteins. In particular, the increased use of 16S rRNA gene sequencing as a diagnostic tool has provided a very powerful means of identifying taxonomically aberrant/atypical strains and recognizing new diversity (e.g. Williams & Collins, 1990; Whiley *et al.*, 1990; Kawamura *et al.*, 1998; Devriese *et al.*, 1997, 1999; Flint *et al.*, 1999; Collins *et al.*, 2000, 2001). Despite a very substantial increase in the number of described streptococcal species in recent years, it is evident that many others remain to be discovered, particularly from human and animal sources. During the course of a study of taxonomically problematic Gram-positive, catalase-negative cocci from veterinary sources, we have used 16S rRNA gene sequencing to characterize a *Streptococcus*-like organism originating

from chickens that does not correspond phenotypically to currently defined species. Based on both phenotypic and phylogenetic evidence, we propose another novel species of the genus *Streptococcus*, *Streptococcus gallinaceus* sp. nov.

Unidentified streptococcal-like organisms were isolated from adult broiler parents (flock size 40 000) with septicaemia. The gross lesions included splenomegaly, hepatomegaly, renomegaly and congestion. Multiple areas of necrosis and/or infarction in the liver and spleen associated with valvular endocarditis were also observed. The aetiological role of the organisms isolated as a poultry pathogen is currently under study, since myeloid leucosis was also observed in the flock. Of several isolates obtained, three cultures (CCUG 42692^T, CCUG 42695 and CCUG 42697) were selected for further characterization. The unidentified isolates were cultured aerobically on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C. The organisms were characterized biochemically by using the API Rapid ID32S and API CORYNE systems according to the manufacturer's instructions (API bioMérieux). Phylogenetic analysis was conducted using 16S rRNA gene sequence analysis. A large fragment of the 16S rRNA gene (corresponding to positions 30 to 1521 of the *Escherichia coli* 16S rRNA gene) was amplified by PCR using conserved primers close to the 3' and 5' ends of the gene. The PCR

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 42692^T is AJ307888.

Fig. 1. Unrooted tree showing the phylogenetic relationships of *Streptococcus gallinaceus* sp. nov. and some other reference streptococcal species. The tree, constructed using the neighbour-joining method, was based on a comparison of approximately 1320 nucleotides. Bootstrap values, expressed as percentages of 200 replications, are given at branching points. Bar, 1%.

products were purified using a Pre-A-Gene kit (Bio-Rad) according to the manufacturer's instructions 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 unidentified 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. 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 and the stability of the groupings was estimated by bootstrap analysis (200 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

Cells of the unidentified isolates recovered from chicken clinical specimens were coccoid in shape, stained Gram-positive and occurred singly, in pairs and in short chains. They were catalase-negative and oxidase-negative and produced α -haemolytic colonies on sheep and horse blood agar. Using commercial API systems, all three isolates produced acid from glucose, lactose, maltose, mannitol, melibiose, methyl β -D-glucopyranoside, pullulan, raffinose, ribose, trehalose and sucrose. The organisms failed to produce acid from D-arabitol, L-arabinose, cyclodextrin, glycogen, melezitose, sorbitol, D-tagatose and D-xylose. All isolates showed activity for alanyl-phenylalanine-proline arylamidase, arginine dihydrolase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase and glycyl-tryptophan arylamidase but no activity was detected for alkaline phosphatase, β -glucuronidase, β -mannosidase, pyroglutamic acid arylamidase or urease. Activity for *N*-acetyl- β -glucosaminidase and pyrazinamidase was either negative or weakly positive.

All isolates hydrolysed aesculin but failed to hydrolyse gelatin and hippurate. The cellular morphology and general biochemical reactions of the isolates were consistent with their assignment to the genus *Streptococcus*, but they did not correspond to any currently known species. To assess the phylogenetic position of the unknown coccus-shaped organisms, their 16S rRNA gene sequences were determined and subjected to a comparative analysis. Over 1400 bases were determined for each isolate and pair-wise analysis revealed 99.8–100% sequence similarity, demonstrating their high genotypic relatedness. Sequence searches of the GenBank and RDP libraries revealed that the unknown bacterium (as exemplified by strain CCUG 42692^T) was phylogenetically most closely associated with streptococcal species (data not shown). A tree constructed by neighbour-joining depicting the phylogenetic affinity of the unknown coccus within the genus *Streptococcus* is shown in Fig. 1. The unknown bacterium formed a distinct subline, clustering within a small subgroup that includes *Streptococcus acidominimus*, *Streptococcus ovis* and *Streptococcus suis*.

It is evident from the polyphasic taxonomic study that the unidentified organisms recovered from clinical specimens from chickens represent an unknown member of the genus *Streptococcus*. Phylogenetically, the unknown bacterium displayed an affinity with *S. ovis*, a species associated with clinical specimens from sheep (Collins *et al.*, 2001). However, a sequence divergence of 5% and a relatively low bootstrap resampling value (56%) indicated that this association was not particularly significant. Other close relatives of the unidentified bacterium included *S. acidominimus* and *S. suis*. It is pertinent to note that Chatellier *et al.* (1998) have shown the latter species to be heterogeneous. In particular, several serotypes (e.g. serotypes 20, 22, 33) have been shown to be genetically different from the type strain of *S. suis* and related organisms. It is clear, however, from the present study that the unidentified bacterium from chickens does not correspond to either *S. suis* or these other *S. suis*-like organisms (Chatellier

Table 1. Characteristics that are useful in distinguishing *S. gallinaceus* sp. nov. from some closely related species

Abbreviations: β -GAL, β -galactosidase; β -GLUC, β -glucuronidase; MBDG, methyl β -D-glucopyranoside; β -NAG, *N*-acetyl- β -glucosaminidase. Tests were performed using the API Rapid ID32Strep system.

Characteristic	<i>S. acidominimus</i> (<i>n</i> = 1)	<i>S. gallinaceus</i> (<i>n</i> = 3)	<i>S. ovis</i> (<i>n</i> = 6)	<i>S. suis</i> (<i>n</i> = 16)
Acid from:				
Glycogen	—	—	+	+
Mannitol	+	+	+	—
Melibiose	—	+	—	—
MBDG	—	+	—	v
Pullulan	—	+	—	+
Ribose	—	+	—	—
Sorbitol	—	—	+	—
Production of:				
β -GAL	+	+	v	+
β -GLUC	—	—	—	+
β -NAG	—	v	—	v

et al., 1998). In terms of 16S rRNA gene sequence divergence, the chicken bacterium displays more than 3% divergence from *S. acidominimus*, *S. suis* and *S. suis*-like organisms. It is recognized that organisms with 16S rRNA gene sequence divergence values equal to or greater than 3% belong to different species (Stackebrandt & Goebel, 1994). Thus, it is evident from divergence value considerations and the results of the treeing analysis that the unidentified bacterium represents a distinct species. The chicken bacterium is also biochemically unique and can be easily differentiated from other *Streptococcus* species. In particular, the API Rapid ID32S profile 723766(4)43110 readily distinguishes the novel chicken bacterium from its nearest phylogenetic relatives, *S. acidominimus*, *S. ovis* and *S. suis*, and all other streptococcal species described to date. Therefore, based on the findings described, we consider that the chicken bacterium merits classification as a novel species of the genus *Streptococcus*, for which the name *Streptococcus gallinaceus* sp. nov. is proposed. Tests that are useful in distinguishing *S. gallinaceus* from closely related species are shown in Table 1.

Description of *Streptococcus gallinaceus* sp. nov.

Streptococcus gallinaceus (gal.li.na'ce.us. L. n. *gallus* rooster, genus name of the chicken; L. masc. adj. *gallinaceus* pertaining to a domestic fowl).

Cells are Gram-positive, non-spore-forming cocci that occur singly, in pairs or in short chains. Colonies are 0.5–1.0 mm in diameter after 24 h on sheep and horse blood agar. Facultatively anaerobic and catalase- and oxidase-negative. Using commercial API systems, strains produce acid from D-glucose, lactose, maltose, mannitol, melibiose, methyl β -D-glucopyranoside, pul-

lulan, D-raffinose, D-ribose, trehalose and sucrose. Acid is not produced from D-arabitol, L-arabinose, cyclo-dextrin, glycogen, melezitose, sorbitol, D-tagatose or D-xylose. Alanyl-phenylalanine-proline arylamidase, arginine dihydrolase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase and glycyl-tryptophan arylamidase activities are detected. No activity is detected for alkaline phosphatase, β -glucuronidase, β -mannosidase, pyroglutamic acid arylamidase or urease. Variable reactions are obtained for *N*-acetyl- β -glucosaminidase and pyrazinamidase. Aesculin is hydrolysed but gelatin and hippurate are not. Voges–Proskauer test is negative and nitrate is not reduced. The G + C content of the DNA is 40.5 mol%.

Isolated from chickens with sepsis. Habitat is not known. The type strain is CCUG 42692^T (= CIP 107087^T).

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