Streptococcus cecorum, a New Species Isolated from Chickens

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Carboxyphilic strains which had characteristics commonly attributed to the group D streptococci and especially to *Streptococcus bovis* were isolated from the ceca of chickens. However, these strains differed from *S. bovis* in being positive for ribose, melezitose, β -glucuronidase, and alkaline phosphatase, and negative for group D reactions and growth enhancement by CO₂. These differences were confirmed by deoxyribonucleic acid-deoxyribonucleic acid hybridization tests. The strains form a new species, for which the name *Streptococcus cecorum* is proposed. Strain A60 (=NCDO 2674) is the type strain of this species.

During an investigation of the susceptibilities of intestinal bacteria isolated from chickens to antimicrobial agents used in farm animals (G. N. Dutta, D. Vet. Sci. thesis, University of Gent, Gent, Belgium, 1983), a group of carboxyphilic streptococci was found which could not be identified as any known streptococcal species. The strains in this group were able to grow at 45°C and in the presence of 40% bile, two characteristics which are commonly attributed only to the enterococci or group D streptococci. After the tests advocated by Facklam (6) and Sharpe (21) for the separation of fecal streptococci from other streptococci (growth at pH 9.6, in 6.5% NaCl, and at 10 and 45°C) were performed, the strains were tentatively identified as *Streptococcus bovis*. However, three reactions were in discordance with this identification; the strains failed to react with the *Streptococcus* group D antisera used, they were able to grow to a limited extent at pH 9.6, and their growth was definitely better in an atmosphere containing 5% CO₂ than in air.

A subsequent biochemical and genetic study showed that these strains should be designated a new species, which is described here.

MATERIALS AND METHODS

Strains. Strains A2a, A16, A29, A37 through A40, and A60^T (T = type strain) were isolated from the ceca of eight chickens from eight farms in Belgium by using Islam medium (12) supplemented with 30 μ g of neomycin per ml and 15 μ g of nalidixic acid per ml. The plates were incubated for 48 h in 5% CO₂ in air. *S. bovis* CCM 5616 was obtained from the Czechoslovak Collection of Microorganisms, Brno. Other reference strains were obtained from the National Collection of Dairy Organisms (NCDO), Shinfield, Reading, England.

Media and culture incubation. Media were obtained from Oxoid Ltd., Basingstoke, England, unless otherwise stated. Cultures were normally incubated at 37°C.

Growth characteristics. Growth on sheep blood-Columbia agar plates was compared after incubation aerobically and in a 5% CO₂ atmosphere for 16 and 48 h. All of the other tests except the API tests were performed in a 5 to 10% CO₂ atmosphere.

Biochemical reactions. Dextran and levan formation was tested on heart infusion agar supplemented with 5% (wt/vol) sucrose. Acid production was tested in phenol red broth base (GIBCO, Paisley, Scotland) containing 1% (wt/vol) carbohydrate. Glycerol fermentation was tested anaerobically on the solidified medium described above. Reactions were read after 24 and 72 h. Other tests were performed by using API 20 Strep and API 50CH galleries according to the instructions of the manufacturer (API, Basingstoke, England). The galleries were a gift from API. Digestion of starch was tested on Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.).

Physiological test. The hemolysis test and the CAMP reaction test (4) were performed on sheep blood-Columbia agar. Catalase activity was tested with 3% H₂O₂. Reduction of potassium tellurite was tested by using Tellur tablets (Rosco, Taastrup, Denmark). Hippurate hydrolysis (5, 11), reactions in 0.1% methylene blue-milk (5, 24), ammonia production from arginine (17), reduction of triphenyl tetrazolium chloride (6), production of L-tyrosine decarboxylase (20), hyaluronidase, and chondroitin sulfatase (25), pyruvate hydrolysis (10), and gelatinase production (15) were tested by methods described previously.

Resistance tests. Tests for growth at pH 9.6, in 6.5% NaCl, and at 10 and 45°C were performed in duplicate in Todd-Hewitt broth (GIBCO) and in medium A of Barnes et al. (2), and the results were recorded after 24 and 72 h. Survival at 60°C for 30 min was tested by using fully grown cultures in capillary pipettes and inocula diluted 1:100 in tubes containing the prewarmed media described above. Growth on 40% bile was tested on bile-esculin agar, and growth inhibition was tested on Slanetz-Bartley medium, kanamycin

cin-esculin-azide agar, Edwards medium, and mitis-salivarius agar (GIBCO). Resistance to 2.75% KSCN was tested on brain heart infusion agar as described by Skorkovsky (23). Minimum inhibitory concentrations (MIC) of antibiotics were determined by using the agar dilution method and Mueller-Hinton agar (26).

Pathogenicity test. Chickens which were 3 months old were injected intravenously with 1 ml of an overnight Todd-Hewitt broth culture containing approximately 5×10^8 colony-forming units of strain A16, observed for 3 weeks, and then euthanized and necropsied.

Chemotaxonomic studies. Cultures were grown for deoxyribonucleic acid (DNA) preparation, isolation of DNA, determinations of base compositions, and DNA-DNA hybridizations as previously described (6a). For fatty acid analyses, dry cells were degraded by acid methanolysis (16), and fatty acid methyl esters were examined by gas-liquid chromatography as described by Farrow et al. (6a).

Serology. The strains were tested against commercially available antisera.

RESULTS AND DISCUSSION

Biochemical and physiological reactions. The results of the tests which differentiated the cecal isolates from other streptococci isolated from poultry and *S. bovis* are given in Table 1. Other carboxyphilic species of streptococci (e.g., *Streptococcus anginosus*, *Streptococcus constellatus*, *Streptococcus intermedius*, *Streptococcus mitis*, and *Streptococcus morbillorum*) are raffinose and inulin negative; *Streptococcus sanguis* is arginine positive, and *Streptococcus mutans* and "*Streptococcus milleri*" do not grow at 45°C (13, 18, 22). The CO₂-dependent strains from bovine mastitis described by Schwan et al. (19) differ from the cecal isolates by exhibiting negative reactions for inulin, melezitose, raffinose, and ribose. *Streptococcus galinarum* (3) differs from the cecal isolates in its biochemical reactions (Table 1), in exhibiting a group D serological reaction, and in possessing menaquinones and a different fatty acid profile (Farrow et al., in press). Similarly, *S. bovis* may be distinguished from the cecal isolates by its negative ribose, melezitose, β -glucuronidase, and alkaline phosphatase reactions, although this species is poorly defined (8). *Streptococcus uberis* differs in bile resistance, in growth at 45°C, in producing NH₃ from arginine, and in fermenting mannitol and sorbitol but not raffinose (7). Therefore, the cecal isolates are easily distinguished from all of the species of streptococci described above.

Inhibition tests. The results of the MIC tests are shown in Table 2, and these results partially differentiate the cecal strains from *S. bovis*. In addition, the cecal isolates do not grow in 2.75% KSCN, in 0.4% K₂Te₂O₃, on Slanetz medium, or on kanamycin-esculin-azide agar. Small colo-

nies appeared on Edwards medium after 2 days, and well-developed slimy light blue colonies formed on mitis-salivarius agar.

Pathogenicity test. The cecal strains were non-pathogenic in the infection model which we used.

DNA base composition. The DNAs base of the cecal isolates contained 37 to 38 mol% guanine plus cytosine (G+C); these values are similar to values reported previously for *Streptococcus faecalis* (9), *Streptococcus faecium* (9), *S. mutans* (9), and *S. bovis* (9). Under the same conditions, *Leuconostoc mesenteroides* NCDO 768 DNA had a G+C content of 39 mol%, and *Escherichia coli* K-12 NCDO 1984 DNA had a G+C content of 51 to 52 mol%.

DNA-DNA hybridization. The specific activity of ³H-labeled DNA from strain A60^T was approximately 3,300 cpm/ μ g. The cecal isolates formed a single homology group that was 82 to 102% related to strain A60^T. None of the four species having similar DNA base compositions was related to strain A60^T. The homology values for *S. bovis* strains NCDO 597^T, NCDO 599, NCDO 2019, NCDO 2571, and NCDO 2594 were 29, 31, 35, 22, and 28% respectively; the homology values for *S. faecalis* NCDO 581^T, *S. faecium* NCDO 942^T, and *S. mutans* NCDO 2062^T were 21, 16, and 19%, respectively. *Streptococcus* sp. strain NCDO 2137, which resembles *S. bovis* biochemically (Farrow, unpublished data) was 24% related to strain A60^T. All the results given above were obtained by using optimum hybridization conditions (melting temperature minus 25°C).

Fatty acid analyses. The 3 cecal isolates examined possessed predominantly straight-chain saturated and monounsaturated fatty acids (Table 3). The major fatty acids corresponded to hexadecanoic (C_{16:0}) and octadecenoic (C_{18:1}) acids. Cyclopropane ring acids were either absent or present in only trace amounts (Table 3). It is worth noting that *S. bovis* and related strains also possess major amounts of C_{16:0} and C_{18:1} acids and generally lack cyclopropane ring acids (J. A. E. Farrow, A. J. Bramley, J. Kruze, B. A. Phillips, and M. D. Collins, manuscript in preparation). However, the absence of lactobacillic acid (C_{19:} Δ) in the cecal isolates clearly distinguishes them from *S. faecalis* and *S. faecium*, which possess substantial levels (10 to 15%) of this acid (Farrow et al., in press).

The data given above indicate that the cecal isolates belong to a new species of the genus *Streptococcus*. Both the DNA-DNA hybridization data and the fatty acid profiles suggest a closer relationship to *S. bovis* than to the enterococci or oral streptococci. DNA-ribosomal ribonucleic acid hybridization of these strains with representatives from these groups (9, 14) may

TABLE 1. Characteristics that differentiate *S. cecorum* from *S. bovis* and other streptococci isolated from poultry^a

Characteristic	<i>S. cecorum</i> (cecal isolates)	<i>S. bovis</i>	" <i>S. avium</i> "	<i>S. faecalis</i>	<i>S. faecium</i>	<i>S. galli-</i> <i>narum</i>	<i>S. pleo-</i> <i>morphus</i>
Growth in the presence of O ₂	+ ^b	+	+	+	+	+	-
Growth at 10°C	-	-	+ or - ^c	+	+	+	-
Growth at pH 9.6	w	± or - ^c	+	+	+	+	-
Survival at 60°C for 30 min	-	-	+	+	+	D	-
Growth in 6.5% NaCl	-	-	+ or w ^c	+	+	+	-
Arginine hydrolysis	-	-	± or - ^c	+	+	-	-
Hippurate hydrolysis	-	-	-	+	±	+	-
Pyrrolidonylarylamidase	-	-	+	+	+	+	-
α-Galactosidase	± ^e	+	-	-	-	+	-
β-Glucuronidase	+	-	-	-	-	+	-
β-Galactosidase	± ^e	-	-	-	+	+	-
Alkaline phosphatase	+	-	-	-	-	-	-
Group D antigen	-	+	+	+	+	+	-
Acid from:							
Glycerol	-	-	±	+	± or - ^c	±	-
L-Arabinose	-	-	±	-	+	+	-
Ribose	+	-	+	+	+	+	-
D-Xylose	-	-	-	+	+	+	-
Adonitol	-	-	+	-	-	-	-
Galactose	+	+	+	+	+	+	+
Sorbose	-	-	+	-	-	-	-
Rhamnose	-	-	±	±	±	-	-
Mannitol	-	±	+	+	+ or ± ^c	+	-
Sorbitol	-	-	+	+	-	-	-
α-Methyl-D-mannoside	-	-	±	-	±	-	-
α-Methyl-D-glucoside	-	-	+	-	±	+	-
Salicin	+	+	+	+	+	+	-
Cellobiose	+	+	+	+	+	+	-
Maltose	+	+	+	+	+	+	-
Lactose	+	+	+	+	+	+	-
Melibiose	+	+	+ or ± ^c	-	± or + ^c	+	-
Sucrose	+	+	±	±	+	+	-
Inulin	+	±	-	-	±	±	-
Melezitose	+	-	+	+	-	-	-
D-Raffinose	+	+	-	-	±	+	-
Starch	± ^d	+	-	-	-	-	-
Glycogen	± ^d	±	-	-	-	-	-
Xylitol	-	-	±	-	-	-	-
D-Turanose	-	-	-	-	±	+	-
D-Lyxose	-	-	+	-	-	-	-
D-Tagatose	± (w) ^d	-	+	+	±	+	-
D-Arabitol	-	-	+	-	-	-	-
L-Arabitol	-	-	±	-	-	-	-
Gluconate	-	-	±	+	±	+	-
2-Ketogluconate	± (w) ^e	-	+	-	-	-	-
5-Ketogluconate	± (w) ^e	-	-	-	-	-	-

^a Data from references 1, 3, 6, 13, and 21 and unpublished data.^b +, ≥90% positive; -, ≤10% positive; ±, >10 <90% positive; w, usually weak reactions. These percentages may not be reliable in the case of *S. cecorum* and "*S. avium*" because they are based on results obtained with a small number of strains.^c Conflicting data have appeared in the literature.^d Type strain NCDO 2674 is positive.^e Type strain NCDO 2674 is negative.

establish the exact taxonomic status of the new species within the genus *Streptococcus* as currently defined. The new species is formally proposed below.

Streptococcus cecorum sp. nov. (ce.co'rum. L. n. *cecum* sacculated diverticulum of the large

intestine [cecum]; N.L. gen pl. n. *cecorum* of ceca) cells are gram-positive cocci 1 to 1.3 µm in diameter and occur in pairs, short chains, or small groups. Within the chains and groups the cells are arranged in pairs. The cells are nonmotile and nonsporing. Colonies are circular with

TABLE 2. MICs of selective substances and antibacterial agents for *S. cecorum* and *S. bovis*

Substance	MIC range ($\mu\text{g/ml}$)	
	<i>S. cecorum</i>	<i>S. bovis</i> CCM 5614
K-Tellurite	250–500	500
Tetrazolium	250–500	500
Sodium azide	500–2000	4,000
Crystal violet	1–2	4
Acriflavine	4	1
Thallium acetate	1,024–>2,048	1,024
Penicillin G	<0.12–2	
Tetracycline	1–64 ^a	
hydrochloride		
Erythromycin	0.5–>128 ^a	
Lincomycin	<0.12–>128 ^a	
Sulfamethoxazole	>256	
Monensin	<1–>128 ^a	
Bacitracin	<0.12–32 ^a	
Carbadox	0.25–2	
Avoparcin	1–2	
Flavomycine	<0.25–0.25	

^a Some strains have acquired resistance.

entire edges, low convex, partially translucent, and not pigmented and reach 1 to 2 mm in diameter after 20 h and 2 to 3 mm after 2 days at 37°C in CO₂ in air. Growth is slower and less abundant in air. The strains show good growth anaerobically in an H₂-CO₂ atmosphere. Uniform turbidity with a small deposit is formed in brain heart infusion broth and Todd-Hewitt broth. Fermentative metabolism. Gas is not formed from glucose. Good growth occurs at 45°C, but no growth occurs at 10°C, in 6.5% NaCl, in 2.75% KSCN, in 0.4% K₂Te₂O₃, on Slanetz medium, or on kanamycin-esculin-azide agar. Growth at pH 9.6 is slower and less abundant than growth at pH 7.0. Slight inhibition by 40% bile. Strains do not survive 60°C for 30 min. Hemolytic on sheep blood agar, producing greening. Strains are negative in the CAMP reaction test, in tests for ammonia from arginine, pyrrolidonylarylamidase, and gelatinase, tyrosinase, and in conventional and API hippurate tests. The majority of the strains react in rapid hippurate tests (only strain A16 is negative). Tetrazolium is not reduced. Dextran and levan are not formed. Hydrolysis of starch is variable; two strains (strains A6 and A28) are amylase positive in the Mueller-Hinton starch digestion test, but only strain A37 does not react in the API-amidon test. Esculin is hydrolyzed. Alkaline phosphatase, leucine arylamidase, β -glucuronidase, and Voges-Proskauer positive. α - and β -galactosidase tests are variable; only strains A16 and A39 are positive in these tests. Most strains produce hyaluronidase and chondroitin sulfatase (only strain A16 is negative in these two tests). Variable and not easily repro-

TABLE 3. Fatty acid composition of *S. cecorum*

Strain	Fatty acid composition (%)					
	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{19:0}
NCDO 2669	2.0	35.0	4.5	13.5	45.0	
NCDO 2671	2.5	38.5	3.5	14.5	41.0	
NCDO 2674 ^T	2.0	27.5	3.5	9.5	47.0	0.5

ducible reactions occur in methylene blue-milk. Acid is formed from *N*-acetylglucosamine, amygdalin, arbutin, cellobiose, fructose, galactose, glucose, β -gentiobiose, inulin, lactose, maltose, mannose, melezitose, melibiose, raffinose, ribose, salicin, sucrose, and trehalose. Variable reactions with glycogen (strains A29 and A60^T react positively). Some weak reactions occur with tagatose, 2-ketogluconate, and 5-ketogluconate. Acid is not formed from adonitol, D- or L-arabinose, D- or L-arabitol, dulcitol, erythritol, fucose, α -methyl-D-glucoside, glycerol, inositol, lyxose, mannitol, α -methyl-D-mannoside, L-rhamnose, sorbitol, L-sorbose, turanose, xylitol, xylose, or β -methyl-xyloside. The DNA base composition ranges from 37 to 38 $\pm$ 1 mol% G+C. The long-chain fatty acids are straight-chain saturated and monounsaturated acids. The major fatty acids are hexadecanoic and octadecenoic (ω 7) acids. Lactobacillic acid is either absent or is present in only trace amounts.

The eight strains of *S. cecorum* have been deposited in the National Collection of Dairy Organisms as strains NCDO 2667 through 2674. The type strain is strain NCDO 2674 (=A60). The reactions of strain NCDO 2674^T are those of the majority of the strains, but this strain has acquired tetracycline (MIC, 16 $\mu\text{g/ml}$), erythromycin (MIC, >128 $\mu\text{g/ml}$), lincomycin (MIC, >128 $\mu\text{g/ml}$), and bacitracin (MIC, 4 $\mu\text{g/ml}$) resistances. The DNA base composition of strain NCDO 2674^T is 37 mol% G+C.

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