Taxonomic Study of Lancefield Streptococcal Groups C, G, and L (*Streptococcus dysgalactiae*) and Proposal of *S. dysgalactiae* subsp. *equisimilis* subsp. nov.

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Streptococcus dysgalactiae consists of at least five distinct subgroups on the basis of serogroups, biotypes, and hosts. A chemotaxonomic and phenotypic examination of 80 *S. dysgalactiae* strains representing the known diversity within this species and 49 reference strains representing all members of the streptococcal pyogenic species group revealed two subpopulations of strains within *S. dysgalactiae*. The name *S. dysgalactiae* subsp. *dysgalactiae* is proposed for strains of animal origin. These strains belong to Lancefield serogroups C and L, are alpha-, beta-, or nonhemolytic, and do not exhibit streptokinase activity on human plasminogen or proteolytic activity on human fibrin. The name *S. dysgalactiae* subsp. *equisimilis* is proposed for human isolates. These strains belong to Lancefield serogroups C and G, are beta-hemolytic, and exhibit streptokinase activity on human plasminogen and proteolytic activity on human fibrin.

In 1936, Frost and Engelbrecht proposed the name *Streptococcus equisimilis* for a group of beta-hemolytic streptococci belonging to Lancefield serogroup C (10, 12). *S. equisimilis* strains were isolated from the nose, throat, vagina, and skin of humans and were thought to be uncommon in domestic animals (2). About a decade later, the bovine organism *Streptococcus dysgalactiae* was reported to be identical to *S. equisimilis*, except for the absence of beta-hemolysis (2). However, in 1980, both species names lost standing in nomenclature when they were not included on the Approved Lists of Bacterial Names (15). *S. dysgalactiae* was revived in 1983, but was restricted to the alpha-hemolytic, group C strains of bovine origin (11). Subsequently, Farrow and Collins (9) demonstrated that *S. dysgalactiae*, *S. equisimilis*, and streptococci belonging to serogroups G (the large-colony-forming strains) and L all exhibited high levels of DNA-DNA binding and therefore belonged to a single species, *S. dysgalactiae*. Except for *Streptococcus agalactiae* (serogroup B), the serological groups designed by Lancefield (12) do not correspond to individual species and for species other than *S. agalactiae* are useful primarily for differentiating infraspecific biovars or ecovars (5). In the case of *S. dysgalactiae*, serotyping made species level identification extremely complex. Among the group C streptococci, the following five taxa have been differentiated: (i) the human, beta-hemolytic strains; (ii) the porcine, beta-hemolytic strains previously named *S. equisimilis*, which belong to *S. dysgalactiae*; (iii) the bovine, alpha-hemolytic strains, which are the *S. dysgalactiae* strains defined by Garvie et al. (11); (iv) the equine, beta-hemolytic strains which belong to the two subspecies of *Streptococcus equi* (*S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus*); and (v) the human, small-colony-forming, beta-hemolytic strains which belong to the “*Streptococcus milleri*” group (3, 5). Among the group G streptococci, the following three taxa have been differentiated: (i) the human, large-col-

ony-forming, beta-hemolytic strains which belong to *S. dysgalactiae*; (ii) the human, small-colony-forming, beta-hemolytic strains which belong to the “*S. milleri*” group; and (iii) the bovine, canine, and feline, beta-hemolytic strains, which belong to *Streptococcus canis* (6). Finally, as explained above, the beta-hemolytic group L streptococci also belong to *S. dysgalactiae* (9).

Although invalid, the name *S. equisimilis* is still widely used (3, 7, 13), and the nomenclature of the entire group remains confused. In order to shed light on the taxonomy of these organisms, we examined 80 *S. dysgalactiae* strains representing the various subgroups within this species and 49 additional reference strains by using whole-cell protein electrophoresis, a technique that has been shown to be very useful for differentiating strains at or below the species level (4). The phenotypic characteristics of the clusters of strains obtained were extensively examined, and a new classification for these bacteria is proposed.

MATERIALS AND METHODS

Bacterial strains. Type and other reference strains were obtained from international culture collections as listed in Table 1. The other strains examined were mostly our field isolates (isolates of L.A.D.). All of the strains used and their sources are listed in Table 1. Bacteriological purity was checked by plating and examining living and Gram-stained cells.

Whole-cell protein analysis. All strains were grown for 24 h on brain heart infusion agar (catalog no. 0037-17-8; Difco Laboratories, Detroit, Mich.) incubated at 36 to 37°C in a microaerobic atmosphere containing approximately 5% O₂, 10% CO₂, and 85% N₂. Preparation of cellular protein extracts, polyacrylamide gel electrophoresis (PAGE), a densitometric analysis, normalization and interpolation of the protein profiles, and a numerical analysis were performed as described by Pot et al. (14) by using the GelCompar 3.1 software package (Applied Maths, Kortrijk, Belgium). The profiles were recorded and stored on a PC computer. The levels of similarity between pairs of traces were expressed by the Pearson product moment correlation coefficient converted for convenience to a percentage.

Physiological tests. Strains were grown on Columbia agar (Lab M, Paisley, United Kingdom) supplemented with 5% cattle blood at 37°C in an atmosphere containing 5% CO₂ in air. Serological grouping with Lancefield group A, B, C, D, F, and G antigens was carried out by using an Oxoid streptococcal grouping kit (Unipath, Basingstoke, United Kingdom). Acid (0.2 N HCl) Lancefield extracts of strains that did not react in these tests were tested by using group L antiserum obtained from D. G. Groothuis (National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands).

Streptokinase activity was determined on fibrin-plasma plates made up from

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TABLE 1. Strains used

Name	LMG no. ^a	Other designation(s) ^a	Depositor ^{a,b}	Source and/or group
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	14602	Ton 516		Bovine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	14604	Ton 517		Bovine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	14605	Ton 528		Bovine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	14606	Higgins 79	Higgins	Chicken, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15732	STR 656		Porcine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15733	STR 655, CCUG 27659		Porcine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15734	STR 654, CCUG 27658		Porcine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15735	STR 653, CCUG 27657		Porcine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15736	T3583	Hommez	Porcine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15737	T3588	Hommez	Porcine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15743	94/1479		Iguana, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15744	Ton 332		Porcine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15748	STR 240		Bovine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15749	STR 490		Bovine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15750	STR 273		Bovine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15751	STR T64	Hommez	Bovine, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15752	STR T12	Hommez	Bovine, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15753	STR 449, CCUG 27666		Bovine, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15754	Higgins 58	Higgins	Chicken, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15755	Higgins 67	Higgins	Chicken, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15756	STR 772		Porcine, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15757	STR 771		Porcine, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15758	Ton 130		Canine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15759	Ton 133		Canine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15766	Ma R		Bovine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15824	CCUG 28112	CCUG	Equine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15830	CCUG 11671A	CCUG	Canine, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15831	CCUG 27665	CCUG	Canine, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15832	CCUG 27666	CCUG	Bovine, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15833	CCUG 28114	CCUG	Equine uterus, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15834	CCUG 28115	CCUG	Equine blood, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15835	CCUG 28116	CCUG	Canine vagina, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15836	CCUG 28120	CCUG	Porcine joint, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15896	STR 785		Canine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15897	STR 782		Canine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15899	CCUG 28113	CCUG	Porcine abscess, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15900	CCUG 28117	CCUG	Porcine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15901	CCUG 28118	CCUG	Equine throat, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15902	CCUG 28119	CCUG	Equine abscess, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	15931	STR 284		Canine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	16023 ^T	NCFB 2023 ^T , LMG 15885 ^T	NCFB	Bovine, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	16024	NCFB 2024	NCFB	Bovine mastitis, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	16025	NCFB 2043	NCFB	Bovine milk, group C
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	16027	NCFB 2648	NCFB	Animal, group L
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	16029	NCFB 2652	NCFB	Canine, group L
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15738	API 79.12.012	API	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15739	API 81.10.028	API	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15740	API 84.11.160	API	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15745	API 83.02.040	API	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15746	API 79.01.052	API	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15747	API 86.04.100	API	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15818	CCUG 1845	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15819	CCUG 4211	CCUG	Human vagina, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15820	CCUG 18742	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15821	CCUG 20596	CCUG	Human blood, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15822	CCUG 27621	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15823	CCUG 27622	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15825	CCUG 32016	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15826	CCUG 32017	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15828	CCUG 32019	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15829	CCUG 27483	CCUG	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15837	CCUG 32201	CCUG	Human blood, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15839	CCUG 33802	CCUG	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15840	CCUG 1483	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15841	CCUG 6713	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15842	CCUG 21557	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15843	CCUG 27478	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15844	CCUG 27479	CCUG	Human, group C
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15845	CCUG 27480	CCUG	Human, group C

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TABLE 1—Continued

Name	LMG no. ^a	Other designation(s) ^a	Depositor ^{a,b}	Source and/or group
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15846	CCUG 502	CCUG	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15847	CCUG 1859	CCUG	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15848	CCUG 7975	CCUG	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15849	CCUG 15679	CCUG	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15850	CCUG 15680	CCUG	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15851	CCUG 24070	CCUG	Human throat, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15852	CCUG 26147	CCUG	Human wound, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15853	CCUG 27477	CCUG	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15854	CCUG 27482	CCUG	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	16026 ^T	NCFB 1356 ^T	NCFB	Human, group G
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	15903	CCUG 28238	CCUG	Human throat, group C
<i>S. agalactiae</i>	14694 ^T	CCUG 4208 ^T	CCUG	Bovine milk
<i>S. agalactiae</i>	14747	STR 765		Bovine mastitis
<i>S. agalactiae</i>	14847	SHV 357	Hommez	Porcine
<i>S. agalactiae</i>	15084	18RS21	Melin	Human
<i>S. agalactiae</i>	15085	M732	Melin	Human
<i>S. agalactiae</i>	15095	455889	Melin	Human, respiratory infection
<i>S. anginosus</i>	14502 ^T	CCUG 27298 ^T	CCUG	
<i>S. anginosus</i>	14612	STR 778		Canine
<i>S. anginosus</i>	14613	STR 200		Canine
<i>S. anginosus</i>	14696	CCUG 223	CCUG	
<i>S. anginosus</i>	15994	CCUG 28467	CCUG	Human
<i>S. canis</i>	14833	CCUG 27660, STR 290		Canine
<i>S. canis</i>	14834	STR 642		Feline
<i>S. canis</i>	15890 ^T	STR-T1 ^T		Bovine mastitis
<i>S. canis</i>	15891	21650-1/473		Mink
<i>S. canis</i>	15892	03342-1106		Fox
<i>S. canis</i>	15893	20837/7820		Fox
<i>S. canis</i>	15894	23668/2140		Mink
<i>S. equi</i> subsp. <i>zooepidemicus</i>	15760	Ton6		Equine
<i>S. equi</i> subsp. <i>zooepidemicus</i>	15761	P11264		Equine
<i>S. equi</i> subsp. <i>zooepidemicus</i>	15762	P13997		Equine
<i>S. equi</i> subsp. <i>zooepidemicus</i>	15763	Ton 174		Equine
<i>S. equi</i> subsp. <i>equi</i>	15764	STR 674		Equine
<i>S. equi</i> subsp. <i>equi</i>	15886 ^T	CCUG 23255 ^T	CCUG	Equine
<i>S. equi</i> subsp. <i>zooepidemicus</i>	15888	CCUG 23257	CCUG	
<i>S. equi</i> subsp. <i>zooepidemicus</i>	15889	CCUG 23258	CCUG	
<i>S. equi</i> subsp. <i>zooepidemicus</i>	16030 ^T	NCFB 1358 ^T , LMG 15887 ^T	NCFB	Bovine mastitis
<i>S. hyointestinalis</i>	14579 ^T	S93 ^T		Porcine
<i>S. hyointestinalis</i>	14581	S85		Porcine
<i>S. hyointestinalis</i>	14582	EV 28b		Porcine
<i>S. iniae</i>	14520 ^T	CCUG 27303 ^T	CCUG	Dolphin
<i>S. iniae</i>	14521	CCUG 27623	CCUG	Dolphin
<i>S. parauberis</i>	12173	NCFB 2018, LMG 14377	NCFB	Bovine mastitis
<i>S. parauberis</i>	12174 ^T	NCFB 2020 ^T , LMG 14376 ^T	NCFB	Bovine mastitis
<i>S. porcinus</i>	14615	STR 827		Human
<i>S. porcinus</i>	15980 ^T	CCUG 27628 ^T	CCUG	Porcine
<i>S. porcinus</i>	15981	CCUG 27629	CCUG	Porcine
<i>S. pyogenes</i>	14237	CIP 56.58	CIP	Patient with rheumatic fever, throat
<i>S. pyogenes</i>	14238	CIP 70.3	CIP	Patient with bronchopneumonia, pleural fluid
<i>S. pyogenes</i>	14700 ^T	CCUG 4207 ^T	CCUG	
<i>S. pyogenes</i>	15855	A4260	Goossens	Human throat
<i>S. pyogenes</i>	15856	A6825	Goossens	Human throat
<i>S. pyogenes</i>	15857	H1667	Goossens	Human throat
<i>S. pyogenes</i>	15858	C8185	Goossens	Human throat
<i>S. pyogenes</i>	15859	P4091	Goossens	Human throat
<i>S. shiloi</i>	15978 ^T	CIP 103769 ^T	CIP	Fish, brain

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TABLE 1—Continued

Name	LMG no. ^a	Other designation(s) ^a	Depositor ^{a,b}	Source and/or group
<i>S. uberis</i>	14395	STR 192		Duck, lung
<i>S. uberis</i>	14610	STR 203		Bovine
<i>S. uberis</i>	14686	ATCC 13386	ATCC	

^a API, Appareils et Procédés d'Identification, La-Balme-les-Grottes, Montalieu-Vercieu, France; ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; CIP, Collection bactérienne de l'Institut Pasteur, Paris, France; Goossens, H. Goossens, University Hospital Antwerp UIA, Antwerp, Belgium; Higgins, R. Higgins, Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Montreal, Sainte-Hyacinthe, Canada; Hommez, J. Hommez, Regional Veterinary Investigation Centre, Torhout, Belgium; LMG, Laboratorium voor Microbiologie Gent Culture Collection, Universiteit Ghent, Gent, Belgium; Melin, P. Melin, Centre Hospitalier Universitaire de Liège, Service de Microbiologie Médicinale, Liège, Belgium; NCFB, National Collection of Food Bacteria, Agricultural and Food Research Council, Institute of Food Research, Reading Laboratory, Reading, Berkshire, United Kingdom.

^b Our isolate if not indicated otherwise.

Columbia blood agar base supplemented with 1 g of bovine fibrinogen fraction I (catalog no. F-8630; Sigma) per liter and 0.5% human plasma. Fibrinogen (100 mg) was first dissolved in 10 ml of phosphate-buffered saline and warmed for 10 min in a shaking water bath at 37°C. This preparation was then mixed with 100 ml of melted blood agar base and incubated for 10 min at 56°C to allow for precipitation of fibrin. Plates were poured immediately after plasma was added and were stored for a maximum of 3 days. A plate containing the same base and bovine fibrin without plasma was used as a control. In other tests, canine, porcine, equine, bovine, and avian (chicken) plasmas were used as plasminogen sources at the same concentration. A limited number of strains giving positive or negative results on Columbia agar supplemented with bovine fibrin and plasma were also tested on brain heart infusion agar (Oxoid, Basingstoke, United Kingdom) and on nutrient agar (Oxoid) similarly supplemented with human plasma and bovine fibrin. Strains were spot inoculated onto the plates, and clearing of bovine fibrin on plates containing plasma and not on plates containing only fibrin was considered evidence of activation of plasminogen to plasmin (streptokinase) activity. Reactions occurring on plasma-free as well as on plasma-supplemented plates were interpreted as evidence of fibrinolysis caused by protease activity. This activity was confirmed by tests on casein plates (Columbia agar supplemented with 0.5% skim milk) and gelatin plates (Columbia agar supplemented with 0.15% Bacto Gelatin [Difco]). Another test involved the use of Columbia agar made up from its separate components but with starch omitted (see below). Tests for protease activity on fibrin from other mammal species (humans, horses, and dogs) were also carried out. Fibrin plates prepared with fibrinogens from human plasma (catalog no. F-4129; fraction I, type III; Sigma), from horse plasma (catalog no. F-9516; fraction I; Sigma), and from dog plasma (catalog no. F-7128; Sigma) as described above were used, but plasma was not added.

RESULTS

PAGE of whole-cell proteins. A total of 129 streptococci were examined. Duplicate protein extracts of several strains were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. The level of correlation between duplicate protein patterns was at least 94% (data not shown). After visual comparison of the protein patterns with the dendrogram obtained after numerical comparison and clustering of the profiles, we identified 12 stable clusters above a similarity level of 84% (Fig. 1). Cluster I comprised 45 *S. dysgalactiae* strains, including type strain LMG 16023, which clustered above a similarity level of 84.8%. These strains were isolated from various animals and belong to serogroups C and L (Fig. 1; Table 1). Cluster III comprised 35 *S. dysgalactiae* strains that clustered above a similarity level of 84.7%. All of the cluster III strains were isolated from human sources and belong to serogroups C and G (Fig. 1; Table 1). As shown in Fig. 1, the reference strains of all of the other streptococcal species examined constituted homogeneous clusters, as follows: *S. canis* (cluster II; grouping above a similarity level of 87.2%), *Streptococcus pyogenes* (cluster IV; grouping above a similarity level of 88.9%), *Streptococcus uberis* (cluster V; grouping above a similarity level of 84.1%), *S. agalactiae* (cluster VI; grouping above a similarity level of 85.8%), *Streptococcus hyointestinalis* (cluster VII; grouping above a similarity level of 91.7%), *Streptococcus parauberis* (cluster VIII; grouping above a similarity level of 96.3%), *Streptococcus porcinus*

(cluster IX; grouping above a similarity level of 88.7%), *Streptococcus anginosus* (cluster X; grouping above a similarity level of 89.2%), and *S. equi* (cluster XI; grouping above a similarity level of 88.4%). Cluster XII comprised the two *Streptococcus iniae* reference strains and the type strain of *Streptococcus shiloi*, which grouped above a correlation level of 93.2%. Figure 2 shows the whole-cell protein patterns of representative strains belonging to different clusters.

Physiological tests. Physiological tests were performed on all 80 *S. dysgalactiae* strains. The serotypes of these strains according to the Lancefield scheme are shown in Table 1. All of the strains except the bovine group C strains which were isolated from tonsils (strains LMG 14602, LMG 14604, and LMG 14605) and milk (strains LMG 15748, LMG 15749, LMG 16024, LMG 16025, and LMG 16023^T [T = type strain]) and a single group C strain isolated from a pig (strain LMG 15744) exhibited beta-hemolysis on bovine blood agar. The bovine group C strains were alpha-hemolytic or nonhemolytic. Porcine strain LMG 15744 was also alpha-hemolytic.

All 35 strains belonging to cluster III as determined by the whole-cell protein analysis (Fig. 1) produced streptokinase activity on human plasminogen. This was shown by the presence of neat and easily visible zones of clearing surrounding streptococcal growth spots on bovine fibrin-plasma plates. Most reaction zones were visible after 1 day of incubation, but in a few cases, when growth was weak, they appeared only after 2 days. No strain reacted with plasminogens from the five animal species tested. The strains also did not cause fibrinolysis on bovine fibrin plates without plasma, and they were all negative in protease tests on milk agar and gelatin agar. The amylase reaction, which is visible with the naked eye on Columbia agar base without fibrin, did not interfere with the streptokinase tests because the starch contained in the medium was hydrolyzed and could not be demonstrated, even with iodine, following the addition of the fibrin preparations to the Columbia agar base. Tests performed with laboratory-prepared Columbia agar without starch gave the same results.

One equine protein electrophoretic cluster I strain (LMG 15824) produced some clearing on bovine fibrin-horse plasma plates. This clearing was visible beneath the growth spot only when the spot was washed off after 2 days of incubation. None of the 44 other cluster I strains tested, including the 4 other equine Lancefield group C strains, gave evidence of streptokinase activity in the plate tests. All of these strains except the eight bovine group C strains listed above were negative in tests performed on bovine fibrin plates with or without different types of plasma, including human plasma, and on milk agar and gelatin agar. These strains lacked streptokinase activity. However, the eight alpha- or nonhemolytic bovine group C strains (including the *S. dysgalactiae* type strain) produced ex-

FIG. 1. Dendrogram derived from unweighted pair group average linkage of correlation coefficients (expressed for convenience as percentages) for the whole-cell protein patterns of all of the strains examined. Roman numerals are cluster numbers (see text).

actly the same fibrinolysis zones on bovine fibrin plates with and without human or animal plasma. This indicates that the lysis observed was not due to plasmin activity resulting from the action of streptokinase on plasminogen, but was caused by

proteolytic enzymes. Indeed, the same strains lysed casein on milk agar plates; however, they were not active on gelatin.

All of the cluster III strains produced neat zones of fibrinolysis on human fibrin-containing plates without added plasma,

FIG. 2. Protein profiles of representative strains of each electrophoretic cluster. For *S. agalactiae*, *S. anginosus*, and *S. porcinus*, protein patterns of both human and animal isolates are shown (Table 1). The molecular weight markers used (lane A) were (from left to right) lysozyme (molecular weight, 14,500), trypsin inhibitor (20,100), trypsinogen (24,000), carbonic anhydrase (29,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), egg albumin (45,000), and bovine albumin (66,000).

while none of the cluster I strains exhibited any visible activity on this substrate. On canine fibrin plates, seven of the eight bovine cluster I strains produced wide zones of lysis which were identical to the zones that they produced on bovine fibrin agar plates and on milk agar plates. The remaining strains were not active on this type of fibrin. All of the strains studied were negative on horse fibrin.

DISCUSSION

Selection of *S. dysgalactiae* strains. *S. dysgalactiae* consists of at least five distinct subgroups on the basis of serogroups, biotypes, and hosts (5, 7). In the present study, the sources of the strains used included humans, horses, cows (and milk), pigs, dogs, chickens, and an iguana (Table 1). These isolates represented nonhemolytic, alpha-hemolytic and beta-hemolytic *S. dysgalactiae* strains and serogroups C, G, and L. Thus, the strains used (Table 1) reflect the known diversity within this species.

Differentiation of streptococci by whole-cell protein electrophoresis. *S. agalactiae*, *S. canis*, *S. dysgalactiae*, *S. equi*, *S. hyointestinalis*, *S. iniae*, *S. parauberis*, *S. porcinus*, *S. pyogenes*, and *S. uberis* constitute the pyogenic species group as determined by 16S rRNA sequence analysis (1). Other streptococcal species are more remotely related to members of this group. In the present study, we included representative strains of all of these taxa as reference strains. In addition, *S. anginosus* was also included, as this species also comprises Lancefield group C and G strains. *S. dysgalactiae* strains were easily differentiated from other streptococci by whole-cell protein electrophoresis (Fig. 1 and 2). However, these organisms fell into two major clusters, with all of the animal isolates constituting one cluster (cluster I) (Fig. 1) and the human isolates constituting the other (cluster III) (Fig. 1). Clusters I and III contain five (LMG 16023^T through LMG 16025, LMG 16027, and LMG 16029) and one

(LMG 16026^T) reference strains, respectively, which were included in extensive DNA-DNA hybridization studies (9) (Fig. 1; Table 1). The DNA-DNA binding values obtained by using the membrane filter technique between these strains and other *S. dysgalactiae* reference strains ranged from 66 to 100% (9), indicating that they form a homogeneous species. As determined by protein electrophoresis, these strains are representative of their respective clusters, and we therefore concluded that the two clusters indeed represent a genotypically coherent species despite salient protein electrophoretic differences. These differences occur mainly in the 45,000- to 55,000-molecular-weight region (molecular weights were estimated roughly after comparison with the molecular weight ladder [Fig. 2]). These two clusters apparently represent different electrophoretic types of *S. dysgalactiae*, and the origins of the *S. dysgalactiae* strains apparently are useful for identifying the electrophoretic subgroups. The collection of 80 strains examined in this study was selected in such a way that it represented the biochemical and serological diversity within the species, and we assume that it is therefore representative of the entire species. From an epidemiological point of view, these findings indicate that humans are never or only rarely infected by *S. dysgalactiae* strains from animals. Interestingly, the animals from which strains have been isolated are not only farm animals, but also companion animals. Consequently, not only farm workers but also pet owners are regularly exposed to *S. dysgalactiae* strains of animal origin, yet are not clinically infected. The improved method for distinguishing between animal-associated and human-associated *S. dysgalactiae* strains described here should help trace possible zoonotic infections.

The reference strains of *S. agalactiae*, *S. anginosus*, *S. canis*, *S. equi*, *S. iniae*, *S. hyointestinalis*, *S. parauberis*, *S. porcinus*, *S. pyogenes*, and *S. uberis* constitute separate clusters on the dendrogram (Fig. 1). The *S. shiloi* type strain forms a single cluster

TABLE 2. Characteristics that differentiate *S. dysgalactiae* subspecies

Taxon	Hemolysis	Lancefield carbohydrate antigens	Streptokinase activity on human plasminogen	Proteolysis of human fibrin	Acid production from ^a :		α -L-Glutamate aminopeptidase activity ^a	L-Prolyl-L-arginine aminopeptidase activity ^a
					Sorbitol	Glycogen		
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	Alpha, beta, or none	C, L	— ^b	—	D	D	D	+
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	Beta	C, G	+	+	—	—	+	D

^a Data from reference 7.^b —, negative; +, positive; D, strain dependent.

with the *S. iniae* strains at a correlation level of 93.2%, confirming the reported synonymy of these two species (8). As illustrated in Fig. 1 and 2, *S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus* cannot be differentiated by whole-cell protein electrophoresis. In contrast to the *S. dysgalactiae* strains, human and animal isolates of *S. agalactiae*, *S. anginosus*, and *S. porcinus* do not seem to exhibit significant differences in their whole-cell protein patterns (Table 1; Fig. 2).

Phenotypic differentiation of *S. dysgalactiae* strains. Despite the superficial resemblance of the assay methods, direct proteolytic activity on human fibrin and streptokinase activity on human plasminogen are two different characteristics which can be used as easy ways to distinguish between human and animal isolates of *S. dysgalactiae* in diagnostic laboratories. McCoy and coworkers (13) found that streptokinases of different species obtained from different hosts were antigenically related. In their study, all of the group C strains tested, including strains referred to as *S. equisimilis*, *S. equi* subsp. *equi*, and *S. equi* subsp. *zooepidemicus*, had surface receptors that bound human, equine, and porcine plasmins. Human *S. equisimilis* strains were active only on human plasminogen, and this finding was confirmed and extended in the present study with avian, bovine, and canine plasmas. However, equine *S. equisimilis* strains and strains from the two *S. equi* subspecies activated horse plasma, while porcine *S. equisimilis* strains were active on porcine plasminogen and, very weakly, also on human plasminogen. In the study of McCoy et al. (13), plasminogen activator activity was identified by hydrolysis of a plasmin-specific chromogenic substrate. This was detected in growth patches by a solid-phase assay. In our study, the enzyme was detected by lysis of fibrin, the normal substrate of plasmin. The difference in test results may be due to differences in the sensitivities of the two test systems.

Taxonomic status of *S. dysgalactiae* strains of human origin and animal origin. Species level identification of *S. dysgalactiae* strains is complex and provides little information for clinicians or epidemiologists. Beta-hemolytic group L *S. dysgalactiae* strains and alpha-hemolytic group C *S. dysgalactiae* strains colonize or infect only animals, whereas beta-hemolytic group G *S. dysgalactiae* strains colonize or infect only humans. Beta-hemolytic group C *S. dysgalactiae* strains occur in humans and animals, but the animal isolates do not occur in humans and vice versa. Clearly, from a practical point of view, *S. dysgalactiae* consists of two distinct subpopulations of strains, which produce very different whole-cell protein electrophoretic patterns, as demonstrated in Fig. 1. The protein electrophoretic differences are comparable to the differences between distinct species in the group containing the pyogenic streptococci (Fig. 1 and 2). In contrast, human and animal isolates of several other members of this species group do not differ significantly (Fig. 1 and 2; Table 1). The two subpopulations of *S. dysgalactiae* can also be readily identified by phenotypic tests, as described above. In our opinion, these observations warrant

distinct taxonomic status for each subpopulation of strains. As Farrow and Collins (9) demonstrated that both of the subpopulations belong to a single species, we propose that they should be classified as distinct subspecies of *S. dysgalactiae*. The name *S. dysgalactiae* subsp. *dysgalactiae* is used for the subpopulation of strains of animal origin because this subpopulation includes the type strain. Historically, the name *S. equisimilis* was given to human isolates resembling *S. equi* strains which occurred in animals, and therefore it is logical to use the name *S. dysgalactiae* subsp. *equisimilis* for the *S. dysgalactiae* strains of human origin. In addition, this avoids creating more confusion by the introduction of a new name.

S. dysgalactiae subsp. *dysgalactiae* strains can be distinguished from *S. dysgalactiae* subsp. *equisimilis* strains by proteolysis of human fibrin, by the results of the human plasminogen-streptokinase test, by origin, and by whole-organism protein electrophoretic patterns. Lancefield serogrouping is also of value for differentiating the two subspecies; group L streptococci always belong to *S. dysgalactiae* subsp. *dysgalactiae*, while group G streptococci of human origin belong to *S. dysgalactiae* subsp. *equisimilis* (all animal group G strains described to date belong to *S. canis*). These identifications can be confirmed by physiological tests (6, 7). Also, for group C streptococci, origin can be used for presumptive identification; however, the strains should first be differentiated from *S. equi* and "*S. milleri*" (7, 9). Finally, Efstratiou et al. (7) described additional phenotypic tests that can be used to differentiate the two subspecies (Table 2).

Description of *S. dysgalactiae* subsp. *dysgalactiae* subsp. nov. The description of *S. dysgalactiae* subsp. *dysgalactiae* is the same as the description given by Farrow and Collins (9) for *S. dysgalactiae*, with following exceptions. Most strains are beta-hemolytic, but alpha-hemolytic and nonhemolytic strains occur, especially in bovine sources. Strains may react with Lancefield group C or L antigen but not with Lancefield group G antigen. Streptokinase activity on human plasminogen and proteolytic activity on human fibrin do not occur. The habitat is the respiratory and genital tracts of various animals, but apparently not humans.

Description of *S. dysgalactiae* subsp. *equisimilis* subsp. nov. The description of *S. dysgalactiae* subsp. *equisimilis* is the same as the description given by Farrow and Collins (9) for *S. dysgalactiae*, with following exceptions. Strains are beta-hemolytic and may react with Lancefield group C or G antigen but not with Lancefield group L antigen. Streptokinase activity occurs on human plasminogen, and proteolytic activity occurs on human fibrin. The habitat is the respiratory tracts and vaginas of humans. The type strain is LMG 16026 (= NCFB 1356), which belongs to Lancefield group G.

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