JOURNAL OF CLINICAL MICROBIOLOGY, July 1990, p. 1497–1501
0095-1137/90/071497-05$02.00/0

Vol. 28, No. 7

Phenotypic Differentiation of *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* Strains within the "*Streptococcus milleri* Group"

R. A. WHILEY,[1] H. FRASER,[1] J. M. HARDIE,[1] AND D. BEIGHTON[2]*

Department of Oral Microbiology[1] and Hunterian Dental Research Unit,[2] The London Hospital Medical College, Turner Street, Whitechapel, London E1 2AD, United Kingdom

Received 9 February 1990/Accepted 10 April 1990

A biochemical scheme was developed by which strains of *Streptococcus constellatus*, *Streptococcus intermedius*, and *Streptococcus anginosus* can reliably be distinguished from within the "*Streptococcus milleri* group." Strains identified as *S. intermedius* were differentiated by the ability to produce detectable levels of α-glucosidase, β-galactosidase, β-D-fucosidase, β-*N*-acetylgalactosaminidase, β-*N*-acetylglucosaminidase, and sialidase with 4-methylumbelliferyl-linked fluorogenic substrates in microdilution trays after 3 h of incubation at 37°C, together with the production of hyaluronidase. Strains of *S. constellatus* and *S. anginosus* were differentiated by the production of α-glucosidase and hyaluronidase by the former and the production of β-glucosidase by the latter. The majority of strains of the *S. milleri* group obtained from dental plaque were identified as *S. intermedius*, as were most strains isolated from abscesses of the brain and liver. Strains of *S. constellatus* and *S. anginosus* were from a wider variety of infections, both oral and nonoral, than were strains of *S. intermedius*, with the majority of strains from urogenital infections being identified as *S. anginosus*.

The term "*Streptococcus milleri* group" has been used to describe a biochemically, serologically, and genetically heterogeneous collection of strains that includes streptococci referred to variously as *Streptococcus milleri* (10, 17), *Streptococcus* MG (25), the minute-colony-forming streptococci of Lancefield groups F and G (8, 22), *Streptococcus intermedius* (19), *Streptococcus constellatus* (19), *Streptococcus anginosus* (1, 12), *Streptococcus* MG-*intermedius* (14), and *Streptococcus anginosus-constellatus* (14). Such strains are currently classified within a single species, *Streptococcus anginosus* (12).

Although the *S. milleri* group forms part of the normal flora of the human oral cavity and upper respiratory, gastrointestinal, and female urogenital tracts (2, 22, 24, 25, 31, 33, 37), such organisms are frequently isolated from purulent infections of the mouth and internal organs, including the brain, liver, lungs, and spleen (4, 9, 13, 23, 27, 35, 38), and from cases of appendicitis, peritonitis, endocarditis, meningitis, obstetric and neonatal infections, and infections of the skin and soft tissues (21, 28, 30, 35, 38). Evidence for the pathogenicity of these streptococci has recently been extensively reviewed (16, 32).

Despite the increasing awareness of the clinical significance of these streptococci, their taxonomy and nomenclature have remained unresolved; consequently, there is much confusion in the literature (12, 15, 18, 32). Previously, Coykendall et al. (12) reported that the *S. milleri* group consists of only one species, *S. anginosus*. However, in a recent study of 25 strains of the *S. milleri* group, we demonstrated the presence of three genetic groups (39): DNA homology group 1 consisted of 5 strains and included the type strain of *S. constellatus* (19), NCDO 2226 (=ATCC 27823), DNA homology group 2 consisted of 4 strains and included the type strain of *S. intermedius* (19), NCDO 2227 (=ATCC 27335), and DNA homology group 3 contained 12 strains and included the type strain of *S. anginosus* (12), NCTC 10713 (=ATCC 12395). These conclusions are different from those of Coykendall et al. (12), most probably because of the respective stringencies of the different methods used (39).

Although these three groups are genetically distinct, there are few reliable phenotypic tests for distinguishing among them (6, 39). In this paper, we report a reliable biochemical scheme by which the three taxa within the *S. milleri* group may be differentiated.

MATERIALS AND METHODS

Bacterial strains. A total of 157 strains of the *S. milleri* group were examined; these included 64 isolates from dental plaque (58 from supragingival plaque and 6 from subgingival plaque from different subjects) and 91 isolates from various other clinical sources. The latter included isolates from oral and nonoral infections of the head and neck (29 strains), pleuropulmonary infections (4 strains), abdominal infections (22 strains), urogenital infections (11 strains), skin and soft tissue infections (13 strains), a musculoskeletal infection (1 strain), blood (7 strains), and urine (4 strains). Two reference strains from culture collections (NCDO 2226 and NCTC 5389) were of unknown origin. All the Lancefield serological grouping data reported in this study were supplied by the donors of the strains.

The collection included the 26 strains previously shown to represent three distinct genetic groups within the *S. milleri* group (39; R. A. Whiley, Ph.D. thesis, University of London, London, England, 1987): DNA homology group 1 contained NCDO 2226 (*S. constellatus* type strain), NCTC 10714, NCTC 11063, NCTC 5389, NCTC 10709, AM699, and UNS 36; DNA homology group 2 contained NCDO 2227 (*S. intermedius* type strain), NMH 2, UNS 35, and 415-87; and DNA homology group 3 contained NCTC 10713 (*S. anginosus* type strain), NCTC 11062, NCTC 8037, ATCC 9895, 2405-81, 2236-81, 1007-77, KR687, KR455, KR533, NMH 10, G5:3, PC4890, B448, and 549.

All strains were stored as freeze-dried cultures and also on

* Corresponding author.

glass beads at −70°C. They were maintained by routine subculturing on Columbia agar (GIBCO Ltd., Paisley, United Kingdom) containing 5% (vol/vol) defibrinated horse blood and were incubated at 37°C in an atmosphere of 20% H_2–10% CO_2–70% N_2.

Biochemical tests. Strains were tested for the ability to degrade the following synthetic fluorogenic substrates: *N-tert*-butoxycarbonyl (BOC)-Leu-Ser-Thr-Arg-7-amido-4-methylcoumarin (AMC), BOC-Val-Leu-Lys-AMC, succinyl-Ala-Ala-Phe-AMC, and BOC-Ile-Glu-Gly-Arg-AMC for the detection of protease activity and 4-methylumbelliferyl (4-MU)-α-L-arabinoside, 2′-(4-MU)-α-D-*N*-acetylneuraminic acid, 4-MU-β-D-cellobioside, 4-MU-β-D-*N*,*N*′-diacetylchitobioside, 4-MU-β-D-*N*,*N*′,*N*″-triacetylchitotriose, 4-MU-*N*-acetyl-β-D-glucosaminide, 4-MU-*N*-acetyl-β-D-galactosaminide, 4-MU-α-L-fucoside, 4-MU-β-D-fucoside, 4-MU-α-D-galactoside, 4-MU-β-D-galactoside, 4-MU-α-D-glucoside, 4-MU-β-D-glucoside, 4-MU-6-sulfo-2-acetamido-2-deoxy-β-D-glucoside (Koch-Light Ltd., Haverhill, United Kingdom), 4-MU-α-L-iduronide (Koch-Light), 4-MU-α-D-mannoside, 4-MU-β-D-mannoside, and 4-MU-β-D-xyloside for the detection of α-L-arabinosidase, sialidase, β-cellobiase, β-diacetylchitobiase, β-triacetylchitotriase, β-*N*-acetylglucosaminidase, β-*N*-acetylgalactosaminidase, α-L-fucosidase, β-D-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, hexosaminidase A, α-iduronidase, α-mannosidase, β-mannosidase, and β-xylosidase activities, respectively. All substrates were purchased from Sigma Chemical Co. Ltd., Poole, United Kingdom, unless otherwise indicated. Substrates were dissolved in a minimum volume of dimethyl sulfoxide and diluted in 50 mM *N*-tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer (pH 7.5) (TES buffer; Sigma) to a final concentration of 100 μg/ml.

Degradation of substrates was determined by growing the bacteria for 2 days in anaerobic jars at 37°C on Fastidious Anaerobe Agar (Lab M, Lancashire, United Kingdom). Colonies were removed with sterile swabs and placed in TES buffer, and the suspension was adjusted to an optical density at 620 nm of 0.1 (approximately 10^8 organisms per ml). Assays were set up by mixing 20 μl of substrate solution with 50 μl of bacterial suspension in a flat-bottomed clear microdilution plate well and were incubated at 37°C for 3 h. Substrate degradation (release of 4-methylumbelliferone or AMC) was visualized by viewing the trays on a UV transilluminator; positive strains produced a blue fluorescence. Trays could also be viewed with a hand-held, long-wavelength UV lamp.

Acid production from amygdalin, lactose, mannitol, and raffinose was tested in microdilution trays by the method of Beighton et al. (5). Strains were tested for esculin hydrolysis and the production of ammonia from arginine as described by Bisset and Davis (7), and acetoin production (Voges-Proskauer test) was determined as described by Cowan (11). Hydrogen peroxide production was tested on plates by the method of Whittenbury (40) with strains incubated in 10% CO_2 in air at 37°C for 4 days. Hyaluronidase production was detected on agar plates containing 400 μg of hyaluronic acid (grade III-S; Sigma) per ml and 1% (wt/vol) bovine serum albumin (fraction V; Sigma) by the rapid method of Smith and Willet (36). Hemolysis was determined by streaking strains onto layered blood agar plates (agar base no. 2; Oxoid Ltd., London, United Kingdom) containing 5% (vol/vol) defibrinated horse blood in the top layer. After incubation at 37°C for 48 h, strains were recorded as hemolytic (beta-hemolysis) or nonhemolytic (alpha- or gamma-hemolysis).

Cluster analysis. The fermentation, physiological, and substrate hydrolysis data were coded as 0 (no reaction) or 1 (positive reaction). Substrates that gave very few positive results or were uniformly positive or uniformly negative were not included in the cluster analysis. The remaining variables were subjected to cluster analysis by using the average-linkage-between-groups method in the SPSS package of statistical programs (26).

RESULTS

Phenotypic test results. All strains produced ammonia from arginine, and virtually all produced acetoin (94%) and hydrolyzed esculin (96%). The fluorogenic substrates (BOC)-Leu-Ser-Thr-Arg-AMC, BOC-Val-Leu-Lys-AMC, succinyl-Ala-Ala-Phe-AMC, BOC-Ile-Glu-Gly-Arg-AMC, 4-MU-α-L-arabinoside, 4-MU-β-D-cellobioside, 4-MU-β-D-*N*,*N*′-diacetylchitobioside, 4-MU-β-D-*N*,*N*,*N*″-triacetylchitotriose, 4-MU-α-L-fucoside, 4-MU-α-D-galactoside, 4-MU-6-sulfo-2-acetamido-2-deoxy-β-D-glucoside, 4-MU-α-L-iduronide, 4-MU-α-D-mannoside, 4-MU-β-D-mannoside, and 4-MU-β-D-xyloside gave either uniformly negative or only weakly positive reactions.

Cluster analysis of all 157 strains carried out on the basis of their reactions in a total of 13 tests (acid production from amygdalin, lactose, mannitol, and raffinose and the production of H_2O_2, hyaluronidase, α-glucosidase, β-glucosidase, β-*N*-acetylglucosaminidase, β-*N*-acetylgalactosaminidase, β-galactosidase, β-D-fucosidase, and sialidase) resulted in the formation of three distinct clusters: cluster A, designated *S. constellatus* (48 strains plus strain NCDO 2226); cluster B, designated *S. intermedius* (60 strains plus strain NCDO 2227); and cluster C, designated *S. anginosus* (46 strains plus strain NCTC 10713). Only four fermentation tests (amygdalin, lactose, mannitol, and raffinose) were included, as previous results obtained with strains in DNA homology groups 1, 2, and 3 had already demonstrated the tests for acid production from arabinose, cellobiose, inulin, glycerol, glucose, salicin, sorbitol, and trehalose to be of no use for differentiating these groups (39).

The results obtained for each of the three clusters in the 13 tests are shown in Table 1. The strains designated *S. intermedius* were easily distinguished from *S. constellatus* and *S. anginosus*, particularly by strongly positive results for the production of β-galactosidase, β-D-fucosidase, β-*N*-acetylglucosaminidase, β-*N*-acetylgalactosaminidase, α-D-glucosidase, sialidase, and hyaluronidase. The production of α-glucosidase, β-glucosidase, and hyaluronidase differentiated between strains of *S. constellatus* and *S. anginosus*. There was complete concordance between the phenotypic clusters obtained in this study and DNA homology groups 1 (*S. constellatus*), 2 (*S. intermedius*), and 3 (*S. anginosus*), previously described (39); each phenotypic cluster contained strains from only one DNA homology group. A scheme for the identification of *S. constellatus*, *S. intermedius*, and *S. anginosus* is shown in Table 2.

The hemolysis and Lancefield grouping reactions of the strains from each phenotypic cluster are shown in Table 3. Strains of *S. anginosus* represented a group displaying considerable serological and hemolytic heterogeneity as compared with strains of *S. intermedius*, which were virtually all nonhemolytic (alpha- or gamma-hemolysis) and serologically ungroupable. Interestingly, of those strains of *S. constellatus* and *S. anginosus* which belonged to Lancefield serological group F, virtually all of the former (13 of 15 strains) were beta-hemolytic, whereas virtually all of the

TABLE 1. Differential phenotypic characteristics of *S. constellatus* (cluster A), *S. intermedius* (cluster B), and *S. anginosus* (cluster C) within the *S. milleri*-group

Test	% of the following positive:		
	S. constellatus (n = 49)	*S. intermedius* (n = 61)	*S. anginosus* (n = 47)
Production of:			
β-D-Fucosidase	0	100	0
β-*N*-Acetylglucosaminidase	0	100	0
β-*N*-Acetylgalactosaminidase	0	100	0
Sialidase	0	100	0
β-Galactosidase	2	100	0
β-Glucosidase	4	47	96
α-Glucosidase	90	100	19
Hyaluronidase	88	98	4
H_2O_2	6	0	34
Acid from:			
Amygdalin	29	78	100
Lactose	55	97	98
Mannitol	4	3	17
Raffinose	4	5	32

latter (12 of 13 strains) were nonhemolytic. The results from the fermentation of amygdalin, lactose, mannitol, and raffinose and H_2O_2 production indicated that these tests are not of primary importance for the identification of the three groups.

Colony variation. A total of 17 of 157 (11%) strains produced both rough and smooth colony forms on Columbia agar. These forms showed no differences in biochemical test results on examination. Of these strains, 16 of 17 were *S. intermedius*, with 1 strain being *S. constellatus*, representing 26 and 2% of the strains in these two clusters, respectively.

Distribution of strains. Of the total of 58 strains originating from the supragingival plaque of healthy subjects, 38 (66%) were identified as *S. intermedius*, 14 (24%) were identified as *S. constellatus*, and 6 (10%) were identified as *S. anginosus*. Of the six strains isolated from subgingival plaque, five were identified as *S. constellatus* and one was identified as *S. anginosus*.

The sites of infection from which the remaining 93 strains studied were originally isolated are shown in Table 4. Strains of *S. intermedius* were relatively limited in their distribution, being isolated mainly from brain and liver abscesses. In contrast, strains of *S. constellatus* and *S. anginosus* were isolated from a much wider variety of sites, both oral and

TABLE 2. Differential characteristics of *S. constellatus*, *S. intermedius*, and *S. anginosus*

Characteristic	Result[a] for:		
	S. constellatus	*S. intermedius*	*S. anginosus*
β-D-Fucosidase	−	+	−
β-*N*-Acetylglucosaminidase	−	+	−
β-*N*-Acetylgalactosaminidase	−	+	−
Sialidase	−	+	−
β-Galactosidase	−	+	−
β-Glucosidase	−	d	+
α-Glucosidase	+	+	d
Hyaluronidase	+	+	−

[a] +, 85% or more of the strains were positive; −, 85% or more of the strains were negative; d, 16 to 84% of the strains were positive.

TABLE 3. Hemolysis and Lancefield grouping reactions of strains in each cluster

Lancefield group	% of strains in each cluster with the indicated hemolytic reaction:					
	S. constellatus		*S. intermedius*		*S. anginosus*	
	Beta	Alpha or gamma	Beta	Alpha or gamma	Beta	Alpha or gamma
A	0	2	0	0	0	4
C	0	2	0	0	2	4
F	26	4	0	0	2	26
G	0	0	0	0	8	2
K	0	0	0	3	0	2
L	0	0	0	0	0	2
Nongroupable	12	54	7	90	0	48

nonoral. The majority of strains (11 of 15 [73%]) from urogenital sites were *S. anginosus*, and the remainder were *S. constellatus*. Of these 15 strains, 9 (60%) fermented mannitol or raffinose or both, 13 (87%) were nonhemolytic, 8 (53%) were serologically ungroupable, 6 (40%) belonged to Lancefield group F, and 1 (7%) belonged to Lancefield group C, consistent with the characteristics of strains from these sites reported previously (31, 33).

DISCUSSION

In this study, three phenotypically distinct taxa were demonstrated within a collection of 157 strains belonging to the *S. milleri* group. These clusters each contained members of only one of the three distinct genetic groups previously

TABLE 4. Sites of clinical isolation of strains of *S. constellatus*, *S. intermedius*, and *S. anginosus*

Site or type of infection	No. of the following associated with each infection:		
	S. constellatus	*S. intermedius*	*S. anginosus*
Head and neck			
Dental abscess	4	2	0
Submandibular abscess	3	0	0
Palatal abscess	0	0	3
Root canal	0	0	3
Infected tooth socket	1	0	0
Throat	3	0	3
Nasal cavity	1	0	0
Brain abscess	1	5	0
Pleuropulmonary	2	0	2
Abdominal			
Liver abscess	1	8	0
Appendix	4	1	4
Perforated ulcer	0	0	1
Abdominal abscess	1	1	1
Urogenital	4	0	11
Musculoskeletal	0	0	1
Miscellaneous skin and soft tissue	1	4	8
Blood	4	1	3

demonstrated within the *S. milleri* group (20, 39). These observations provide strong evidence against the inclusion of all strains of the *S. milleri* group within the single species *S. anginosus*, as proposed by Coykendall et al. (12). The clusters reported here were designated *S. constellatus* (cluster A, DNA homology group 1), *S. intermedius* (cluster B, DNA homology group 2), and *S. anginosus* (cluster C, DNA homology group 3), as they included the type strains of these three previously described species.

The phenotypic characteristics of *S. constellatus*, *S. intermedius*, and *S. anginosus* demonstrated that several of the tests frequently used in the presumptive identification and characterization of strains belonging to the *S. milleri* group, namely, hemolytic and Lancefield grouping reactions and lactose fermentation (14, 29, 31–34), are of little practical use for the correct identification of these strains. However, these tests and other biochemical characteristics (e.g., fermentation of amygdalin, mannitol, and raffinose and the production of H_2O_2) may provide useful additional data on strains in clinical and epidemiological studies. Comparisons between the data presented here and the results of previous taxonomic studies of the *S. milleri* group are difficult because of differences in the selection of tests used by various investigators. However, identification of the majority of strains from supragingival plaque as *S. intermedius* is consistent with the findings from previous studies in that strains previously described as *S. milleri* from dental plaque (3, 24, 41) were nonhemolytic and mainly carried no Lancefield group antigen, characteristics which we found to be typical of *S. intermedius* (Table 3).

Several authors (10, 31) have previously reported an association between the production of hyaluronidase and beta-hemolysis activity among *S. milleri* strains. In the present study, this association was observed among *S. constellatus* strains but was not apparent among *S. intermedius* strains, which produced hyaluronidase but were virtually all nonhemolytic.

We found that *S. intermedius* strains were frequently isolated from dental plaque and that this species is more likely than either *S. constellatus* or *S. anginosus* to produce both rough and smooth colony forms on Columbia agar. These observations accord with those of Yakushiji et al. (41), who isolated *S. milleri* strains exhibiting this characteristic from human dental plaque.

The clinical distribution of the strains implies that while *S. intermedius* strains are the most commonly isolated of these three taxa from dental plaque, they are not frequently associated with infections of the oral cavity. However, *S. intermedius* strains appear to be associated with abscesses of the brain and liver. A comprehensive study of a larger collection of strains, cultured from defined clinical infections by the same isolation techniques for all samples irrespective of the nature of the samples, is necessary to confirm this apparent association.

The tests described here are simple and generally rapid to perform. They will permit the reliable identification of *S. constellatus*, *S. intermedius*, and *S. anginosus* strains from clinical specimens and should enable the association of these three species with types of infections to be more accurately assessed.

ACKNOWLEDGMENTS

This study was supported in part by the Medical Research Council (grant GB 8704557).

We thank the following for providing strains: G. Colman, D. Drucker, R. Facklam, P. Handley, M. Kilian, B. Mejare, P. M. Poole, K. Ruoff, E. Shaw, P. Unsworth, W. Wade, and M. Wren.

LITERATURE CITED

1. **Andrewes, F. W., and T. J. Horder.** 1906. A study of the streptococci pathogenic for man. Lancet **ii**:708–713.
2. **Attebury, H. R., V. L. Sutter, and S. M. Finegold.** 1972. Effect of a partially chemically defined diet on human fecal flora. Am. J. Clin. Nutr. **25**:1391–1398.
3. **Ball, L. C., and M. T. Parker.** 1979. The cultural and biochemical characters of *Streptococcus milleri* strains isolated from human sources. J. Hyg. **82**:63–78.
4. **Bateman, N. T., S. J. Eykyn, and I. Phillips.** 1975. Pyogenic liver abscess caused by *Streptococcus milleri*. Lancet **i**:657–659.
5. **Beighton, D., R. R. B. Russell, and H. Hayday.** 1981. The isolation and characterisation of *Streptococcus mutans* serotype h from dental plaque of monkeys (*Macaca fascicularis*). J. Gen. Microbiol. **124**:271–279.
6. **Beighton, D., and R. A. Whiley.** 1990. Sialidase activity of the "*Streptococcus milleri* group" and other viridans group streptococci. J. Clin. Microbiol. **28**:1431–1433.
7. **Bisset, P. J. M., and G. H. G. Davis.** 1960. The microbial flora of the mouth. Heywood, London.
8. **Bliss, E. A.** 1937. Studies upon minute hemolytic streptococci. III. Serological differentiation. J. Bacteriol. **33**:625–642.
9. **Chua, D., H. H. Reinhart, and J. D. Sobel.** 1989. Liver abscess caused by *Streptococcus milleri*. Rev. Infect. Dis. **11**:197–202.
10. **Colman, G., and R. E. O. Williams.** 1972. Taxonomy of some human viridans streptococci, p. 281–299. *In* L. W. Wannamaker and J. M. Matsen (ed.), Streptococci and streptococcal diseases. Academic Press, Inc., New York.
11. **Cowan, S. T.** 1974. Cowan and Steel's manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, Cambridge.
12. **Coykendall, A. L., P. M. Wesbecher, and K. B. Gustafson.** 1987. "*Streptococcus milleri*," *Streptococcus constellatus*, and *Streptococcus intermedius* are later synonyms of *Streptococcus anginosus*. Int. J. Syst. Bacteriol. **37**:222–228.
13. **De Louvois, J.** 1980. Bacteriological examination of pus from abscesses of the central nervous system. J. Clin. Pathol. **33**: 66–71.
14. **Facklam, R. R.** 1977. Physiological differentiation of viridans streptococci. J. Clin. Microbiol. **5**:184–201.
15. **Facklam, R. R.** 1984. The major differences in the American and British Streptococcus taxonomy schemes with special reference to *Streptococcus milleri*. Eur. J. Clin. Microbiol. **3**:91–93.
16. **Gossling, J.** 1988. Occurrence and pathogenicity of the *Streptococcus milleri* group. Rev. Infect. Dis. **10**:257–285.
17. **Guthof, O.** 1956. Uber Pathogene, "vergrunende Streptokokken." Streptokokken-Befunde bei dentogenen Abszessen und Infiltraten im Bereich der Mundhohle. Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg. Abt. 1 Orig. **166**:553–564.
18. **Hardie, J. M.** 1986. Oral streptococci, p. 1054–1063. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
19. **Holdeman, L. V., and W. E. C. Moore.** 1974. New genus, *Coprococcus*, twelve new species, and emended descriptions of four previously described species of bacteria from human feces. Int. J. Syst. Bacteriol. **24**:260–277.
20. **Kilpper-Bälz, R., B. L. Williams, R. Lütticken, and K. H. Schleifer.** 1984. Relatedness of "*Streptococcus milleri*" with *Streptococcus anginosus* and *Streptococcus constellatus*. Syst. Appl. Microbiol. **5**:494–500.
21. **Koepke, J. A.** 1965. Meningitis due to *Streptococcus anginosus* (Lancefield group F). J. Am. Med. Assoc. **193**:739–740.
22. **Long, P. H., and E. A. Bliss.** 1934. Studies on minute hemolytic streptococci; isolation and cultural characteristics of minute beta-hemolytic streptococci. J. Exp. Med. **60**:619–631.
23. **Lütticken, R., U. Wendorff, D. Lütticken, E. A. Johnson, and L. W. Wannamaker.** 1978. Studies on streptococci resembling *Streptococcus milleri* and on an associated surface-protein antigen. J. Med. Microbiol. **11**:419–431.

24. **Mejare, B., and S. Edwardsson.** 1975. *Streptococcus milleri* (Guthof): an indigenous organism of the human oral cavity. Arch. Oral Biol. **20**:757–762.
25. **Mirick, G. S., L. Thomas, E. C. Curnen, and F. L. Horsfall.** 1944. Studies on a non-hemolytic streptococcus isolated from the respiratory tract of human beings. I. Biological characteristics of *Streptococcus M.G.* J. Exp. Med. **80**:391–406.
26. **Norusis, M. J.** 1988. SPSS-PC plus advanced statistics V2.0. SPSS, Inc., Chicago.
27. **Ottens, H., and K. C. Winkler.** 1962. Indifferent and haemolytic streptococci possessing group-antigen F. J. Gen. Microbiol. **28**:181–191.
28. **Parker, M. T., and L. C. Ball.** 1976. Streptococci and aerococci associated with systemic infection in man. J. Med. Microbiol. **9**:275–302.
29. **Poole, P. M., and G. Wilson.** 1976. Infection with minute-colony-forming-β-haemolytic streptococci. J. Clin. Pathol. **29**: 740–745.
30. **Poole, P. M., and G. Wilson.** 1977. *Streptococcus milleri* in the appendix. J. Clin. Pathol. **30**:937–942.
31. **Poole, P. M., and G. Wilson.** 1979. Occurrence and cultural features of *Streptococcus milleri* in various body sites. J. Clin. Pathol. **32**:764–768.
32. **Ruoff, K. L.** 1988. *Streptococcus anginosus* ("*Streptococcus milleri*"): the unrecognized pathogen. Clin. Microbiol. Rev. **1**:102–108.
33. **Ruoff, K. L., and L. J. Kunz.** 1982. Identification of viridans streptococci isolated from clinical specimens. J. Clin. Microbiol. **15**:920–925.
34. **Ruoff, K. L., L. J. Kunz, and M. J. Ferraro.** 1985. Occurrence of *Streptococcus milleri* among beta-hemolytic streptococci isolated from clinical specimens. J. Clin. Microbiol. **22**:149–151.
35. **Singh, K. P., A. Morris, S. D. R. Lang, D. M. MacCulloch, and D. A. Bremner.** 1988. Clinically significant *Streptococcus anginosus* (*Streptococcus milleri*) infections: a review of 186 cases. N.Z. Med. J. **101**:813–816.
36. **Smith, R. F., and N. P. Willett.** 1968. Rapid plate method for screening hyaluronidase and chondroitin sulfatase-producing microorganisms. Appl. Microbiol. **16**:1434–1436.
37. **Unsworth, P.** 1980. The isolation of streptococci from human feces. J. Hyg. **85**:153–164.
38. **Van der Auwera, P.** 1985. Clinical significance of *Streptococcus milleri*. Eur. J. Clin. Microbiol. **4**:386–390.
39. **Whiley, R. A., and J. M. Hardie.** 1989. DNA-DNA hybridization studies and phenotypic characteristics of strains within the '*Streptococcus milleri* group.' J. Gen. Microbiol. **135**:2623–2633.
40. **Whittenbury, R.** 1964. Hydrogen peroxide formation and catalase activity in lactic acid bacteria. J. Gen. Microbiol. **35**:13–26.
41. **Yakushiji, T., M. Katsuki, A. Yoshimitsu, J. Mizuno, and M. Inoue.** 1988. Isolation and physiological characterization of *Streptococcus milleri* strains from human dental plaque. Microbios **55**:161–171.