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Phenotypic characterization of oral streptococci by classical methods

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Abstract

The phenotypic characteristics of 215 strains of oral streptococci were compared on the basis of the results of sorbitol, raffinose and trehalose fermentation, ammonium from arginine production, aesculin hydrolysis, H_2O_2 production, susceptibility to 2 U bacitracin, and growth in 4% NaCl solution. Eleven different species were identified. Among the most noteworthy findings were the low number of H_2O_2-producing strains of *Streptococcus sobrinus*, the effective discrimination between *Streptococcus sanguis* and *Streptococcus mitis* on the basis of raffinose fermentation, and the phenotypic similarity between *Streptococcus oralis* and the former *mutans* group. To avoid the creation of new species, the term biotype is proposed to designate phenotypically distinct organisms which do not qualify as different species.

Introduction

Oral streptococci, which make up a large proportion of the normal flora of the buccal cavity, are involved in the formation of dental caries (Loesche, 1986), can act as the substrate for pathogenic periodontal flora (Liébana and Castillo, 1991), and participate in a number of other diseases (De Louvois, 1980; Facklam, 1977; Gossling, 1988; Moore-Gillon *et al.*, 1981).

Streptococci are isolated in up to 40% of all patients with subacute endocarditis, and are present in some cases of endocarditis occurring in association with a valvular prosthesis (Bayliss *et al.*, 1983; Garrido, 1985; Kramer *et al.*, 1983; Van Hare *et al.*, 1984). These organisms are sometimes collectively known as *viridans* group streptococci.

It is not always possible to assign members of this group to a given serogroup. Moreover, their alpha-haemolytic nature is not a consistent feature among all oral streptococci (Ball and Parker, 1979; Coykendall *et al.*, 1987; Facklam, 1977, 1984; Gossling, 1988; Hardie, 1986; Saunders and Ball, 1980; Wolff and Liljemark, 1978). The variable findings reported by different authors make it difficult to reach a consensus regarding the biochemical properties of oral streptococci, a situation which has led to some confusion regarding the taxonomic status and identification of many organisms.

This study compares the phenotypic features of recently isolated strains of bacteria in an attempt to develop a more straightforward solution to the identification of oral streptococci.

Materials and methods

Isolates

A total of 215 strains of streptococci were isolated from the dental plaque or saliva of different subjects during a 12 month period. Samples of plaque were collected with sterile cotton swabs, and inoculated in MSA (Difco), MSB, MSA base medium plus 1% potassium tellurite (Difco), and bacitracin (Sigma) which was added at a rate of 200 U/ml. The cultures were incubated at 36 ± 1°C for 24 h in anaerobiosis and 24 h in aerobiosis. Compatible colonies were transferred to plates containing Wilkins-Chalgren agar (Difco) and incubated at 36°C for 48 h in anaerobiosis. These cultures were used to prepare inocula of 10^5 to 10^6 cfu/ml for use in biochemical tests. In all assays, the amount of inoculum used was 10 μl.

Identification tests

Sugar and polyalcohol fermentation

The base medium consisted of 15 g trypsin peptone, 5 g yeast extract, 1 g Tween 80, 0.004 g chlorophenol red, 1 g agar, 1,000 ml distilled water and the final pH was 6.4. Sorbitol, raffinose, trehalose and mannitol were added at a final concentration of 1%. Tubes containing 3 ml of medium were inoculated, incubated at 36 ± 1°C and read at 24 and 48 h. A positive reading was recorded when the culture turned yellow or when the pH decreased by more than 0.5 units on the pH meter.

Ammonium production from arginine (ADH)

Tubes containing the medium of Niven *et al.* (1942) were inoculated, covered with sterile paraffin and incubated at 36 ± 1°C for 48 h. Ammonium production was assessed with Nessler reagents (Merck).

Aesculin hydrolysis

Bacteria (10 ml) were inoculated by stabbing in medium consisting of 10 g peptone, 1 g ammonium ferric citrate, 1 g aesculin, 0.5 g agar and 1,000 ml distilled water (final pH 7.3—7.4). The tubes were incubated at 36 ± 1°C for 24 h, and recorded as positive when a black colour was visible throughout the length of the tube.

H_2O_2 production

Trypticase soy agar (TSA) (Difco) with 1 ml of a 1 mg/ml methanolic solution of orthodianisidine (Merck) was used as the base medium, which was spread in Petri dishes and inoculated. The plates were incubated for 48 h at 36 ± 1°C in anaerobiosis, then for 5 h at the same temperature in aerobiosis. Hydrogen peroxide production was assessed by adding 5 μl of 1 mg/ml horseradish peroxidase solution (Sigma); the appearance of an orange colour was interpreted as a positive result.

Table 1 Results of the biomedical tests used to diagnose oral streptococci

Species	Strains tested	Biochemical tests: H_2O_2	Sorbitol	Raffinose	Mannitol	Trehalose	ADH	Aesculin	NaCl	Bacitracin
S. mutans	30	0 (0)	30 (100)	30 (100)	30 (100)	30 (100)	0 (0)	30 (100)	3 (10)	30 (100)
S. sobrinus	18	3 (16.6)	15 (83.3)	3 (16.6)	18 (100)	18 (100)	0 (0)	0 (0)	15 (83.3)	12 (66.6)
S. cricetus	4	0 (0)	4 (100)	4 (100)	4 (100)	4 (100)	0 (0)	4 (100)	4 (100)	0 (0)
S. macacae	4	0 (0)	0 (0)	0 (0)	4 (100)	4 (100)	0 (0)	0 (0)	4 (100)	0 (0)
S. rattus	4	0 (0)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)
S. mitior	28	28 (100)	0 (0)	25 (88.5)	0 (0)	9 (32.1)	0 (0)	0 (0)	0 (0)	13 (46.9)
S. mitis	20	20 (100)	3 (15)	0 (0)	0 (0)	16 (80)	20 (100)	11 (55)	5 (25)	11 (55)
S. salivarius	34	0 (0)	2 (5.8)	14 (41.1)	0 (0)	10 (29.4)	0 (0)	16 (47)	8 (25.5)	7 (41.1)
S. sanguis	38	38 (100)	14 (36.8)	38 (100)	0 (0)	29 (76.8)	38 (100)	29 (76.8)	5 (13.1)	10 (26.8)
S. milleri	29	0 (0)	0 (0)	0 (0)	0 (0)	17 (58.5)	29 (100)	15 (51.7)	10 (34.4)	28 (96.5)
S. oralis	6	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	0 (0)	0 (0)	0 (0)	0 (0)

H_2O_2 production, fermentation of sorbitol, raffinose, mannitol and trehalose, ammonium production from arginine (ADH), aesculin hydrolysis, growth in the presence of 4% NaCl, and resistance to 2 U bacitracin. The percentage of strains yielding positive results is given in parentheses.

Bacitracin susceptibility

To the base medium used to detect fermentation of sugars and polyalcohols we added 1% glucose and 2 U/ml bacitracin. The tubes were inoculated, incubated at 36 ± 1°C and read after 24 h. A change in colour from red to yellow, and a decrease in pH, were interpreted as showing resistance of the strain to bacitracin.

Growth in 4% NaCl

Two sets of assays were run in tubes containing 3 ml of one of the media described below. In one assay the medium was the same as that described in the previous section for bacitracin resistance, but with 4% NaCl in place of the antibiotic. The inoculated tubes were incubated at 36 ± 1°C for 48 h, after which a positive result was recorded in that there was a colour change or a decrease in pH. The medium for the second assay was trypticase soy broth (Difco) with 4% NaCl. The inoculated tubes (10 μl) were incubated at 36 ± 1°C for 48 h, and the results were considered positive when bacterial growth was observed.

Results

The results of the biochemical tests are given in Table 1, and Table 2 summarizes the proposed identification criteria for oral streptococci. Figure 1 is a simplified identification scheme based on the present results. The reference strains used and their phenotypic characteristics are shown in Table 3.

Table 2 Biochemical diagnosis of oral streptococci

Species	Biochemical tests: H_2O_2	S	R	M	T	ADH	A	NaCl	Bacitracin
S. mutans	−	+	+	+	+	−	+	V−	+
S. sobrinus	V−	V+	V−	+	+	−	−	V+	V+
S. cricetus	−	+	+	+	+	−	+	+	−
S. macacae	−	−	−	+	+	−	−	+	−
S. rattus	−	+	+	+	+	+	+	+	−
S. mitior	+	−	V+	−	V−	−	−	−	V−
S. mitis	+	V−	−	−	V+	+	V+	V−	V−
S. salivarius	−	V−	V−	−	V−	−	V−	V−	V−
S. sanguis	+	V−	+	−	V	+	V+	V−	V−
S. milleri	−	−	−	−	V+	+	V	V−	V+
S. oralis	+	+	+	+	V+	−	−	−	−

H_2O_2, H_2O_2 production; S, R, M, T, fermentation of sorbitol, raffinose, mannitol and trehalose, respectively; ADH, ammonium production from arginine; A, aesculin hydrolysis; NaCl, growth in the presence of 4% NaCl; and Bacitracin, resistance to 2 U bacitracin. −, Negative; +, positive; V−, variable, more than 50% of the strains negative; V+, variable, more than 50% of the strains positive.

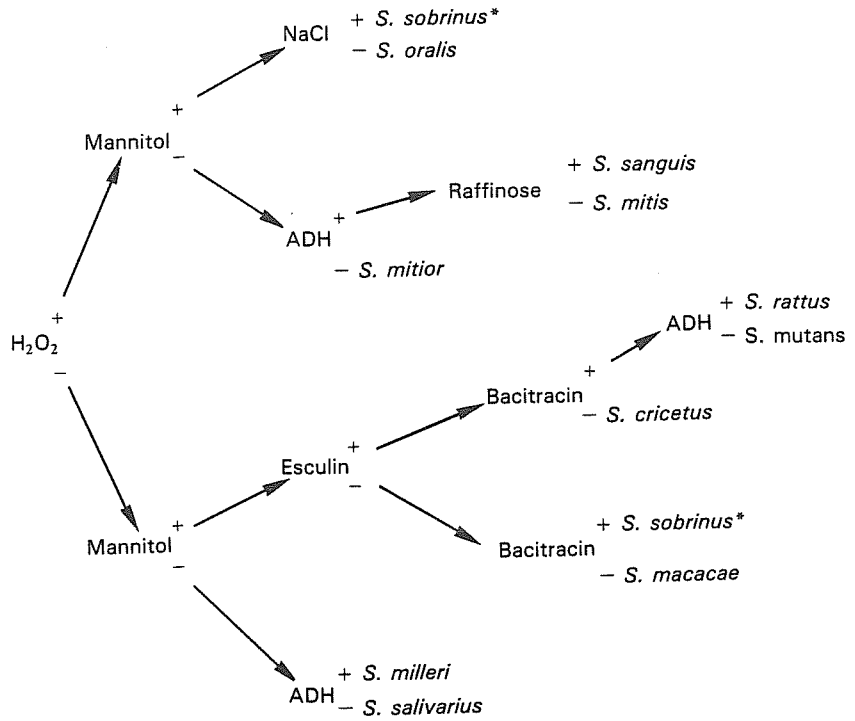

Figure 1 Flow chart for the identification of oral streptococci. *Exceptional strains may fail to grow in the presence of NaCl, and should be checked in Table 2.

Streptococcus mutans showed variable growth in 4% NaCl, failed to produce H_2O_2, and was positive for all other characteristics. *S. sobrinus* consistently fermented mannitol and trehalose, did not hydrolyse aesculin and did not produce ammonium from arginine. It grew well in the presence of 4% NaCl and was frequently resistant to bacitracin. Only a few strains produced H_2O_2. *Streptococcus cricetus*, *S. rattus* and *S. macacae* gave homogeneous results in all biochemical assays.

S. mitior was characterized by hydrogen peroxide production, lack of growth in 4% NaCl, lack of aesculin hydrolysis, lack of ammonium production from arginine, and inability to ferment sorbitol or mannitol. *S. mitis* and *S. sanguis* gave almost identical results with the exception of the production of raffinose acids.

S. milleri did not produce H_2O_2, and did not ferment sorbitol, raffinose or mannitol, but did produce ammonium from arginine. The rest of the tests gave variable results. *S. salivarius* was unable to produce H_2O_2 or ammonium, and did not ferment mannitol. The results of the other tests varied. *S. oralis* was an H_2O_2 producer, and fermented mannitol, sorbitol, raffinose and trehalose. It gave negative results for all other tests.

Table 3 Phenotypical characteristics of the reference strains

Strain	H_2O_2	Sorbitol	Raffinose	Mannitol	Trehalose	ADH	Aesculin	NaCl (4%)	Bacitracin
S. mutans OGS23	−	+	+	+	+	−	+	−	+
S. sobrinus OG342	−	+	−	+	+	−	−	+	+
S. cricetus OGS68	−	+	+	+	+	−	+	+	−
S. macacae OGS200	−	−	−	+	+	−	−	+	−
S. rattus OGS114	−	+	+	+	+	+	+	+	+
S. mitior OGS132	+	−	+	−	−	−	−	−	−
S. mitis OGS2	+	−	−	−	+	+	+	−	+
S. salivarius OGS22	−	−	−	−	−	−	+	−	−
S. sanguis OGS101	+	−	+	−	+	+	+	−	−
S. milleri OGS12	−	−	−	−	+	+	+	−	−
S. oralis OGS25	+	+	+	+	+	−	−	−	−

OGS, Odontological Granada strain. See footnote to Table 2 for explanation of the tests.

Discussion

Oral streptococci are often included in the so-called *viridans* group bacteria, a term introduced by Sherman (1937). *S. morbillorum*, *S. acidominimus*, *S. uberis* and *S. thermophilus* are also normally considered members of this group. It is often difficult to distinguish clearly between members of this group in clinical specimens, although accurate identification is usually a prerequisite for the correct diagnosis of certain infectious diseases. Although a number of tests are based on DNA homology (Beighton *et al.*, 1984; Coykendall *et al.*, 1971; Coykendall *et al.*, 1987) or monoclonal antibodies (Berthold, 1983; De Soet *et al.*, 1987; De Soet *et al.*, 1990; Pekovic *et al.*, 1987), none has proved feasible in routine practice.

Identification of *mutans* group organisms based on serotypes has likewise been unsatisfactory (De Soet *et al.*, 1987). Attempts to base the identity on phenotypical features (Facklam, 1977; Hamada and Slade, 1980; Hardie, 1986; Loesche, 1986; Perch *et al.*, 1974; Whiley *et al.*, 1990) have been hampered by discrepancies between the results of different authors, and so conventional biochemical tests still appear to provide the most reliable means of obtaining a provisional diagnosis in pathological specimens.

Problems in identifying oral streptococci arise from the moment of isolation. Many media of varying degrees of specificity have been described (Carlsson, 1967; Fitzgerald and Adams, 1975; Gold *et al.*, 1973; Ikeda and Sandham, 1972a,b; Linke, 1977; Schaeken *et al.*, 1986; Tanzer *et al.*, 1984; Van Palenstein Helderman *et al.*, 1983), and growth of some *mutans* group species has reportedly been inhibited by MSB (Emilson and Bratthall, 1976; Jordan, 1986; Little *et al.*, 1977; Schaeken *et al.*, 1986; Tanzer *et al.*, 1984; Van Palenstein Helderman *et al.*, 1983; Wade *et al.*, 1986), TYCS and TSY 20 B medium (De Soet *et al.*, 1987).

The approach used in the present study, *i.e.* the use of MSB, a more selective medium, together with MSA, a less selective one, may offer an alternative. Although this method is of little use in qualitative studies of bacteria in dental plaque (De Soet *et al.*, 1987), it is effective in isolating bacteria from other sources, *e.g.* peripheral blood of patients in whom endocarditis is suspected.

We characterized 215 strains of oral streptococci on the basis of the results of nine 'classical' tests. Of these assays sorbitol and trehalose fermentation were found to be uninformative, and can thus be excluded from the panel of tests proposed for an initial diagnosis (Figure 1). Since *S. macacae*, *S. rattus* and *S. cricetus* rarely cause infection in humans, we investigated fewer strains of these species. All showed characteristics similar to those reported by other authors (Hamada and Slade, 1980; Hardie, 1986; Loesche, 1986). *S. mutans* grew in only 10% of the cultures containing 4% NaCl, in contrast with the findings of Hardie (1986), who stated that this species grew consistently in salt-containing media.

Despite the difficulty of identifying strains of *S. sobrinus*, as noted by De Soet *et al.* (1987), the correct identification of these strains is of importance because of their implication in caries formation (De Soet *et al.*, 1989; Lindquist and Emilson, 1989). The aesculin test was negative in our samples, in agreement with the findings of Hamada and Slade (1980), and in contrast with Hardie (1986). This latter author also noted (*op. cit.*) that raffinose fermentation was not a reliable characteristic, in agreement with our finding that only 16.6% of all strains were able to ferment raffinose. However, this contrasts with the results of Hamada and Slade (1980) and Loesche (1986). In our analyses, 66.6% of all *S. sobrinus* strains were resistant to treatment with 2 U of bacitracin, a test formerly considered a reliable indicator for this species (Hardie, 1986; Loesche, 1986).

In addition to the discrepancies between our results and the findings of these authors (*op. cit.*) regarding growth in the presence of NaCl, an even larger divergence was found for H_2O_2 production, which was observed in only 16.6% of our strains. This low figure may have been due to the simultaneous production of large amounts of peroxidase, or to the short period of incubation in aerobiosis (Wittenbury, 1964). However, Quesada (1984) found that 5 h of incubation in aerobiosis was sufficient to obtain valid results for other oral streptococci and similar micro-organisms. Non-peroxide-producing strains susceptible to 2 U bacitracin and unable to grow in medium containing 4% NaCl must be classified either as members of the so-called *mutans* group, or of the species *S. oralis*, since these are the only oral streptococci able to ferment mannitol (Hardie, 1986). They cannot be classified as *S. mutans* since, according to Hamada and Slade (1980), this species produces a positive result in the aesculin test. A diagnosis of *S. cricetus*, *S. macacae* or *S. rattus* is also ruled out since these species are known to be salt-tolerant. Furthermore, *S. rattus* is able to produce ammonium from arginine (Hardie, 1986). *S. oralis* and other candidates were eliminated, because atypical strains of *S. sobrinus* do not simultaneously display the three features mentioned above. Moreover, none of the strains of *S. oralis* is able to grow in the presence of 4% NaCl.

The biochemical characteristics shown by 29 strains indentified as *S. milleri* showed good agreement with the findings of Hardie (1986). This species (or group, according to some authors) encompasses a long list of streptococci (Bliss, 1937; Colman and Williams, 1972; Coykendall *et al.*, 1987; Facklam, 1977; Guthof, 1956; Holdeman and Moore, 1974; Long and Bliss, 1934; Mirick *et al.*, 1944). Recent studies have recommended that *S. intermedius*, *S. constellatus* and *S. anginosus* no longer be included in this group (Beighton and Whiley, 1990; Whiley and Hardie, 1989; Whiley *et al.*, 1990). This further confounds efforts to clarify the identity of oral *Streptococcus* strains, as the strains which we analysed displayed similar responses to biochemical tests, leading us to maintain *S. milleri* as a species designation.

Considerable difficulty remains in distinguishing between *Streptococcus sanguis*, *S. mitior* and *S. mitis*, due to the close similarities in their biochemical behaviour (Cole *et al.*, 1976; Colman and Williams, 1972; Hamada and Slade, 1980; Hardie, 1986; Loesche, 1986; Miller, 1990; Rouff and Ferraro, 1986). Our findings suggest that these three species should stand as such, in view of their differing abilities to produce ammonium and ferment raffinose.

We isolated six different strains of *S. oralis*, a micro-organism that has escaped definitive biochemical characterization (Bridge and Sneath, 1982), showing a bewildering variety of features which potentially relate this bacterium to several different species and groups. Our results, although based on a low number of strains, suggest that phenotypically, *S. oralis* is most closely related to the former *mutans* group.

The 34 strains of *S. salivarius* varied widely in their response to the biochemical tests, with only three features in common: lack of H_2O_2 production, lack of ammonium production, and inability to ferment mannitol. Only 47% of these strains were able to hydrolyse aesculin, leading us to agree with Miller (1990) that the latter test is an unreliable means of identifying *S. salivarius*. In contrast, however, other authors have reported that both the absence (Hamada and Slade, 1980) and the presence (Hardie, 1986) of hydrolysis was a dependable test for this species.

The taxonomic study of oral streptococci continues to be beset by significant problems and gaps, as shown in this study. We used a battery of 'classical' biochemical tests in an attempt to establish a provisional diagnosis of 215 strains isolated from dental plaque. Although we encourage the search for new phenotypical characters which would enhance the precision of future diagnoses, the application of additional features will inevitably further complicate the already complex task of identifying oral streptococci in clinical samples. Attempts to consolidate, rather than further subdivide, the different species of streptococci, should therefore be undertaken with due caution. We recommend that the current number of species be maintained, and that the term biotype be introduced to designate phenotypical differences which are insufficient to justify the creation of new species.

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References

BALL L. C. and Parker M. T. 1979. The cultural and biochemical characters of *Streptococcus milleri* strains isolated from human sources. *J. Hyg. Camb.* **82** 63–78.

BAYLISS R., Clark C., Oakley C. M., Someville W., Whitfield A. G. W. and Young S. E. J. 1983. The microbiology and pathogenesis of infective endocarditis. *Br. Heart J.* **50** 513–9.

BEIGHTON D., Hayday H., Russell R. R. B. and Whiley R. A. 1984. *Streptococcus macacae* sp. nov. from dental plaque of monkeys (*Macaca fascicularis*). *Int. J. Syst. Bact.* **34** 332–5.

BEIGHTON D. and Whiley R. A. 1990. Sialidase activity of the '*Streptococcus milleri* group' and other *viridans* group streptococci. *J. clin. Microbiol.* **28** 1431–3.

BERTHOLD P. 1983. Immunoperoxidase labeling of *Streptococcus mutans* for scanning electron microscopy. *Acta Odontol. Scand.* **41** 125–8.

BLISS E. A. 1937. Studies upon minute hemolytic streptococci. III. Serological differentiation. *J. Bact.* **33** 625–42.

BRIDGE P. D. and Sneath P. H. A. 1982. *Streptococcus gallinarum* sp. nov. and *Streptococcus oralis* sp. nov. *Int. J. Syst. Bact.* **32** 410–5.

CARLSSON J. 1967. A medium for isolation of *Streptococcus mutans. Arch. Oral Biol.* **12** 1657–8.

COLE R. M., Calandra G. B., Huff E. and Nugent K. N. 1976. Attributes of potential utility in differentiating among 'group H' streptococci or *Streptococcus sanguis. J. Dent. Res.* **55** A142–A153.

COLMAN G. and Williams R. E. O. 1972. Taxonomy of some human *viridans* streptococci. *In* Streptococci and Streptococcal Diseases. pp 281–99. Edited by L. W. Wannamaker and J. M. Matsen. Academic Press, New York.

COYKENDALL A. L., Daily O. P., Kramer M. J. and Beth M. E. 1971. DNA-DNA hybridization studies of *Streptococcus mutans. J. Dent. Res.* **50** (Suppl) 1131–9.

COYKENDALL A. L., Wesbecher P. M. and Gustafson K. B. 1987. '*Streptococcus milleri*', *Streptococcus constellatus* and *Streptococcus intermedius* are later synonyms of *Streptococcus anginosus. Int. J. Syst. Bact.* **37** 222–8.

DE LOUVOIS J. 1980. Bacteriological examination of pus from abscesses of the central nervous system. *J. clin. Pathol.* **33** 66–71.

DE SOET J. J., Toors F. A. and De Graaff J. 1989. Acidogenesis by oral streptococci at different pH values. *Caries Res.* **23** 14–7.

DE SOET J. J., Van Dalen P. J., Appelmelk B. J. and De Graaff J. 1987. Identification of *Streptococcus sobrinus* with monoclonal antibodies. *J. clin. Microbiol.* **25** 2285–8.

DE SOET J. J., Van Dalen P. J., Papavicic M. J. A. M. P. and De Graaff J. 1990. Enumeration of *mutans* streptococci in clinical samples by using monoclonal antibodies. *J. clin. Microbiol.* **28** 2467–72.

EMILSON C. G. and Bratthall D. 1976. Growth of *Streptococcus mutans* on various selective media. *J. clin. Microbiol.* **4** 95–8.

FACKLAM R. R. 1977. Physiological differentiation of *viridans* streptococci. *J. clin. Microbiol.* **5** 184–5.

FACKLAM R. R. 1984. The major differences of the American and British *Streptococcus* taxonomy schemes with special reference to *S. milleri. Eur. J. clin. Microbiol.* **3** 91–2.

FITZGERALD R. J. and Adams B. O. 1975. Increased selectivity of mitis-salivarius agar containing polymyxin. *J. clin. Microbiol.* **1** 239–40.

GARRIDO M. 1985. Endocarditis infecciosa. *Med. Clin.* (Barcelona) **64** 655–6.

GOLD O. G., Jordan H. V. and Van Houte J. 1973. A selective medium for *Streptococcus mutans. Arch. Oral Biol.* **18** 1357–64.

GOSSLING J. 1988. Occurrence and pathogenicity of the *S. milleri* group. *Rev. Infect. Dis.* **10** 257–85.

GUTHOF O. 1956. Über pathogene, vergrunende Streptokokken. Streptokokken-Befunde bei dentogenen Abszessen und Infiltraten im Bereich der Mundhole. *Zentbl. Bakt. ParasitKd. Infekt. Hyg.* **1** 533–64.

HAMADA S. and Slade H. D. 1980. Biology, immunology and cariogenicity of *Streptococcus mutans. Microbiol. Rev.* **44** 331–84.

HARDIE J. M. 1986. Genus *Streptococcus*. *In* Bergey's Manual of Systematic Bacteriology. Vol 2, pp 1043–71. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe and J. G. Holt. Williams and Wilkins, Baltimore.

HOLDEMAN L. V. and Moore E. C. 1974. New genus, *Coprococcus*. Twelve new species, and emended descriptions of four previously described species of bacteria from human feces. *Int. J. Syst. Bact.* **24** 260–77.

IKEDA T. and Sandham H. J. 1972a. A medium for the recognition and enumeration of *Streptococcus mutans*. *Arch. Oral Biol.* **17** 601–4.

IKEDA T. and Sandham H. J. 1972b. A high sucrose medium for the identification of *Streptococcus mutans*. *Arch. Oral Biol.* **17** 781–3.

JORDAN H. V. 1986. Cultural methods for the identification and quantitation of *Streptococcus mutans* and lactobacilli in oral samples. *Oral Microbiol. Immunol.* **1** 23–7.

KRAMER H. N., Burgeois M., Liersch R., Nebler L., Meyer H. and Sievers G. 1983. Current clinical aspects of bacterial endocarditis in infancy, childhood, and adolescence. *Eur. J. Pediatr.* **140** 253–9.

LIÉBANA J. and Castello A. 1991. Oral ecology and its pathogenic relation with the origin of primary periodontitis associated with plaque. *Ann. Biol. Clin.* **49** 338–44.

LINDQUIST B. and Emilson C. G. 1989. Distribution of *Streptococcus mutans* and *Streptococcus sobrinus* on tooth surfaces in humans harbouring both species. *Caries Res.* **23** 451.

LINKE H. A. B. 1977. New medium for the isolation of *Streptococcus mutans* and its differentiation from other oral streptococci. *J. clin. Microbiol.* **5** 604–9.

LITTLE W. A., Korts D. C., Thompson A. and Bowen W. H. 1977. Comparative recovery of *Streptococcus mutans* on ten isolation media. *J. clin. Microbiol.* **5** 578–83.

LOESCHE W. J. 1986. Role of *Streptococcus mutans* in human dental decay. *Microbiol. Rev.* **50** 353–80.

LONG P. H. and Bliss E. A. 1934. Studies on minute hemolytic streptococci; isolation and cultural characteristics of minute beta-hemolytic streptococci. *J. exp. Med.* **60** 619–31.

MILLER CH. H. 1990. The oral microbial flora. *In* Oral Microbiology and Infectious Disease. pp 441–64. Edited by G. S. Schuster and B. C. Decker, Philadelphia.

MIRICK G. S., Thomas L., Curnen E. C. and Horsfall F. L. 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.

MOORE-GILLON J. C., Eykin S. J. and Philips I. 1981. Microbiology of pyogenic liver abscess. *Br. Med. J.* **283** 819–21.

NIVEN C. F. Jr, Smiley K. L. and Sherman J. M. 1942. The hydrolysis of arginine by streptococci. *J. Bact.* **43** 651–60.

PEKOVIC D. D., Adamkiewicz V. W., Shapiro A. and Gornitsky M. 1987. Identification of bacteria in association with immune components in human carious dentin. *J. Oral Pathol.* **16** 223–33.

PERCH B., Kjems E. and Ravn T. 1974. Biochemical and serological properties of *Streptococcus mutans* from various human and animal sources. *Acta Path. Microbiol. Scand.* **82** 357–70.

QUESADA A. 1984. Studies of a peroxidase-forming strain of *Streptococcus durans*. Dissertation. University of Granada, Spain.

ROUFF K. and Ferraro M. J. 1986. Presumptive identification of *S. milleri* in 5 hours. *J. clin. Microbiol.* **24** 495–7.

SAUNDERS K. A. and Ball L. C. 1980. The influence of the composition of blood agar on beta haemolysis by *Streptococcus salivarius*. *Med. Lab. Sci.* **37** 341–5.

SCHAEKEN M. J. M., Van der Hoeven J. S. and Franken H. C. M. 1986. Comparative recovery of *Streptococcus mutans* on five isolation media, including a new selective medium. *J. Dent. Res.* **65** 906–8.

SHERMAN J. M. 1937. The streptococci. *Bacteriol. Rev.* **1** 3–97.

TANZER J. M., Borjesson A. C., Laskowki L., Kurasz A. B. and Testa M. 1984. Glucose-sucrose-potassium-tellurite-bacitracin agar for enumeration of *Streptococcus mutans*. *J. clin. Microbiol.* **20** 653–9.

VAN HARE F. G., Ben-Shachar G., Liebman J., Boxerbaum B. and Riemenschneider L. 1984. Infective endocarditis in infants and children during the past 10 years: a decade of change. *Am. Heart J.* **107** 1235–40.

VAN PALENSTEIN HELDERMAN W. H., Ijseldijk M. and Huis H. J. 1983. A selective medium for the two major subgroups of the bacterium *Streptococcus mutans* isolated from human dental plaque and saliva. *Arch. Oral Biol.* **28** 599–603.

WADE W. G., Aldred M. J. and Walker W. M. 1986. An improved medium for isolation of *Streptococcus mutans*. *J. Med. Microbiol.* **22** 319–23.

WHILEY R. A. and Hardie J. M. 1989. DNA-DNA hybridization studies and phenotypic characteristics of strain within the '*Streptococcus milleri* group'. *J. gen. Microbiol.* **135** 2623—33.
WHILEY R. A., Frasser H., Hardie J. M. and Beighton D. 1990. Phenotypic differentiation of *Streptococcus intermedius*, *Streptococcus constellatus* and *Streptococcus anginosus* strain within the '*Streptococcus milleri* group'. *J. clin. Microbiol.* **28** 1497—501.
WITTENBURY R. 1964. Hydrogen peroxide formation and catalase activity in lactic acid bacteria. *J. gen. Microbiol.* **35** 13—26.
WOLFF K. and Liljemark W. F. 1978. Observation of beta hemolysis among three strains of *Streptococcus mutans*. *Infect. Immun.* **19** 745—8.

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