Initial pH as a determining factor of glucose consumption and lactic and acetic acid production in oral streptococci

Article *in* Microbios · February 1996

Source: PubMed

CITATIONS
7

READS
82

5 authors, including:

José Gutiérrez-Fernández
University of Granada
470 PUBLICATIONS **3,933** CITATIONS

SEE PROFILE

Initial pH as a determining factor of glucose consumption and lactic and acetic acid production in oral streptococci

M. L. Concha, A. Castillo, J. Liébana*, J. Gutiérrez and A. García Mendoza

Department of Microbiology, School of Odontology and School of Medicine, University of Granada, Avda de Madrid 11, E-18071 Granada, Spain
(*Reprint address)

Key words: caries, pH, oral streptococci, glucose, lactic acid, acetic acid

Abstract

Lactic and acetic acid production was evaluated from six strains of oral streptococci, *viz Streptococcus gordonii, Streptococcus mutans, Streptococcus oralis, Streptococcus salivarius, Streptococcus sanguis* and *Streptococcus sobrinus* cultured in the presence of 1, 5, 10 mM glucose and without glucose, at initial pH values of 5, 5.5, 6 and 7. *S. sobrinus* and *S. salivarius* caused the greatest decreases in pH. At pH values of 5 and 5.5, lactic acid and acetic acid production in the species tested was discordant with residual glucose levels. Acid production from protein was especially great in *S. mutans* and *S. salivarius*.

Introduction

Dental caries is a multifactorial process influenced by diet, host characteristics, time and micro-organisms. The latter reduce plaque pH, thus triggering demineralization processes (Featherstone *et al.*, 1979).

Oral streptococci the organisms most frequently related to the appearance of dental caries, are acid-producing (acidogenic) bacteria able to grow in an acidic medium (acidophilic), and they continue to produce acids at low pH values (aciduric capacity) as well as synthesize intra- and extracellular polysaccharides (Harper and Loesche, 1984; Loesche, 1986). Since not all oral streptococci display the full range of adaptations to an acidic milieu, their cariogenicity varies from species to species. However, the relation between caries and micro-organisms has been clearly established for the mutans group, which consists of several species (Hamada and Slade, 1980; Hardie, 1986; Loesche 1986; Schleifer *et al.*, 1984), important among which is *Streptococcus sobrinus*, owing to its acidogenic, acidophilic and aciduric properties (DeSoet *et al.*, 1989; Lindquist and Emilson, 1989).

Until genetic differences between oral streptococci are firmly established, their taxonomic positions will continue to be controversial, hence the number of species recognized by different authors (DeSoet *et al.*, 1990; Faclam, 1977; Hamada and Slade, 1980; Hardie, 1986; Loesche, 1986; Whiley *et al.*, 1990).

The present study was designed to investigate the influence of glucose concentration on lactic and acetic acid production, and the effect of initial pH on glucose metabolism, in six species of oral streptococci.

Material and methods

Micro-organisms

The following strains were investigated *Streptococcus gordonii* (OGS 2224), *Streptococcus mutans* (OGS 1024), *Streptococcus oralis* (OGS 1216), *Streptococcus salivarius* (OGS 1424), *Streptococcus sanguis* (OGS 1832) and *Streptococcus sobrinus* (OGS 9121). All strains were obtained in lyophilized form from the Microbiology Laboratory collection of the University of Granada Hospital (OGS, Odontología Granada *Streptococcus*). These strains were identified according to the criteria described by Maiden *et al.* (1992).

Preparation

All strains were incubated on Mitis Salivarius agar (Difco, Detroit, U.S.A). After incubation for a further 24 h under anaerobic conditions at 36 ± 1°C, and for 24 h aerobically at the same temperature, colonies were transferred to trypticase soy broth without glucose (Scott, California, U.S.A.), and incubation continued at 36 ± 1°C. In order to obtain the initial cultures, aliquots were analysed spectrophotometrically at 650 nm (Beckman 25) and the cultures were adjusted, in trypticase soy broth without glucose, by dilution or concentration by centrifugation to an optical density of 1.5, in accordance with the criteria of Gerhardt (1981), who reported this to be equivalent to ~0.55 mg cells (dry wt) per ml, after drying at 105°C. Tubes were prepared with 14 ml trypticase soy broth without glucose, at various pH values (5, 5.5, 6, and 7), obtained with 0.05 M potassium phosphate buffer and 0.05 M citric acid, and verified with a pH meter (Crison Micrograph 2001).

Glucose, which had been sterilized separately to avoid alterations caused by phosphates (Carlsson *et al.*, 1983), was added to each tube at concentrations of 1, 5 or 10 mM. Then 1 ml of the initial culture was added, and the tubes were incubated at 36 ± 1°C for 24 h. Tubes containing broth without glucose were also inoculated, to test for the production of acids from peptides or amino acids in the medium.

Measurement of the final pH

At the end of the last incubation stage, the final pH of the culture medium was measured with a pH meter.

Standard inoculum

A standard inoculum was prepared (Gerhardt, 1981) to avoid inaccurate data due to differential growth of the species tested. Dilution factors were taken into account in the expression of the final results.

Extraction with perchloric acid

The standard inoculum (5 ml) was left in an ice water bath for 5 min, then 5 ml perchloric acid was added to precipitate the proteins. The mixture was shaken by circular orbital motion, and concentrated 0.1 M potassium hydroxide was added until a final pH of 7 was obtained. The amount of potassium hydroxide added was taken into account in the expression of the results. The mixture was cooled for 5 min in an ice water bath, then centrifuged at 10,000 x *g* for 5 min at 0°C. The supernatants were stored at 4°C until use for the remaining analyses.

Evaluation of lactic and acetic acid and residual glucose

Lactic and acetic acid and residual glucose were measured with commercial kits by Boehringer-Mannheim: L-lactic acid 139081, L-acetic acid 148261, and gluco-quant glucose test UV 816434. The procedures and calculations were done according to the manufacturer's instructions. All measurements were performed in triplicate, with a Beckman 25 spectrophotometer.

Results

Tables 1 to 4 summarize the results for initial pH and glucose values, final pH, lactic and acetic acid production, residual glucose, and numbers of bacteria in mg/l (Gerhardt, 1981).

S. gordonii lowered the pH to 4.64 from a starting value of 5.50. Acid production was maximal at pH 7 and a glucose concentration of 10 mM. At pH 5, acid production was lower, and there was always residual glucose.

S. mutans lowered the pH to 4.32 from an initial value of 6.00. Lactic acid production was high at pH values of 6 and 7. In cultures with little or no glucose at an initial pH of 5, considerable amounts of lactic acid were produced, whereas little or no acetic acid was detected.

S. oralis failed to grow at pH 5. The greatest reduction in pH was from an initial value of 6.00 to 4.27. Acid formation was greatest at pH 7 with a glucose concentration of 10 mM, but at lower glucose concentrations acid production was maximal at pH 5.5.

S. salivarius lowered the pH to 4.11 from an initial value of 6.00. Acetic acid production was noted at pH 7. Acid production in relation to the initial glucose concentration was most concordant at pH 7, but at pH 5.5 more acid was produced with 1 and 5 mM glucose that at pH 7. Acid production was considerable in the absence of glucose.

Table 1 The pH, acid production and residual glucose at an initial glucose concentration of 0 mM, in oral streptococci

Species	pH_i	pH_f	ΔpH	Lactic acid (mM)	Acetic acid (mM)	G_f (mM)	Bacteria (mg/l)
S. gordonii	5.0	4.91	0.09	31.01 ± 0.16	34.96 ± 0.15	0	1,447
	5.5	5.51	0.01	48.07 ± 0.20	51.62 ± 0.09	0	1,988
	6.0	5.76	0.24	36.01 ± 0.18	101.57 ± 0.31	0	2,012
	7.0	6.85	0.15	40.44 ± 0.16	26.81 ± 0.10	0	1,398
S. mutans	5.0	4.40	0.60	109.19 ± 0.22	0	0	1,100
	5.5	4.60	0.90	87.19 ± 0.20	1.50 ± 0.10	0	1,375
	6.0	4.76	1.24	86.25 ± 0.19	21.65 ± 0.26	0	1,755
	7.0	6.22	0.78	2.13 ± 0.10	0	0	2,398
S. oralis	5.0	NT	NT	NT	NT	NT	NT
	5.5	4.64	0.86	87.20 ± 0.23	8.33 ± 0.10	0	1,719
	6.0	4.88	1.12	107.46 ± 0.25	43.62 ± 0.22	0	2,426
	7.0	6.48	0.52	13.95 ± 0.20	53.28 ± 0.12	0	3,807
S. salivarius	5.0	4.56	0.44	127.92 ± 0.30	16.65 ± 0.16	0	2,171
	5.5	4.82	0.68	98.23 ± 0.20	5.66 ± 0.12	0	2,426
	6.0	4.76	1.24	125.56 ± 0.51	30.00 ± 0.20	0	2,845
	7.0	6.06	0.94	67.12 ± 0.30	66.61 ± 0.21	0	2,670
S. sanguis	5.0	5.03	0.03	15.27 ± 0.12	0	0	1,684
	5.5	4.79	0.71	64.19 ± 0.22	18.32 ± 0.10	0	2,291
	6.0	5.43	0.57	52.99 ± 0.30	43.96 ± 0.31	0	3,173
	7.0	6.48	0.52	14.52 ± 0.20	66.61 ± 0.21	0	4,230
S. sobrinus	5.0	4.73	0.63	76.64 ± 0.55	2.33 ± 0.10	0	833
	5.5	4.67	0.83	68.06 ± 0.20	35.79 ± 0.30	0	1,355
	6.0	4.98	1.02	89.64 ± 0.23	0	0	1,557
	7.0	6.35	0.65	79.28 ± 0.22	37.63 ± 0.25	0	1,637

pH_i, initial pH; pH_f, final pH; ΔpH, change in pH; G_f, final glucose; means ± standard deviation; NT, not tested.

Table 2 The pH, acid production and residual glucose at an initial glucose concentration of 1 mM, in oral streptococci

Species	pH_i	pH_f	ΔpH	Lactic acid (mM)	Acetic acid (mM)	G_f (mM)	Bacteria (mg/l)
S. gordonii	5.0	4.89	0.11	55.24 ± 0.12	35.97 ± 0.18	1.03 ± 0.11	1,473
	5.5	5.42	0.08	53.66 ± 0.22	44.46 ± 0.22	0	2,357
	6.0	5.60	1.40	52.60 ± 0.15	93.24 ± 0.55	0	2,292
	7.0	6.72	0.28	56.54 ± 0.30	5.99 ± 0.15	0	1,618
S. mutans	5.0	4.56	0.35	48.83 ± 0.25	0	0.13 ± 0.05	645
	5.5	4.47	1.03	107.46 ± 0.14	0	0	1,618
	6.0	4.75	1.25	112.93 ± 0.25	17.15 ± 0.44	0	1,422
	7.0	5.82	1.18	133.20 ± 0.48	6.66 ± 0.13	0	1,825
S. oralis	5.0	NT	NT	NT	NT	NT	NT
	5.5	4.93	0.57	78.71 ± 0.63	10.49 ± 0.12	0	970
	6.0	4.77	1.23	142.62 ± 0.25	33.46 ± 0.59	0	2,750
	7.0	6.22	0.78	29.97 ± 0.30	54.62 ± 0.12	0	3,929
S. salivarius	5.0	4.71	0.29	76.45 ± 0.55	14.82 ± 0.14	0	598
	5.5	4.45	1.05	132.92 ± 0.58	16.81 ± 0.55	0.24 ± 0.10	2,500
	6.0	4.65	1.35	105.10 ± 0.41	0	0.62 ± 0.10	3,173
	7.0	5.88	1.12	107.65 ± 0.30	68.27 ± 0.26	0	2,946
S. sanguis	5.0	4.99	0.01	29.88 ± 0.55	0	0.50 ± 0.01	1,793
	5.5	4.81	0.69	72.77 ± 0.23	19.31 ± 0.52	0	3,330
	6.0	5.24	0.76	98.98 ± 0.22	103.91 ± 0.55	2.30 ± 0.10	3,300
	7.0	6.28	0.72	41.85 ± 0.13	41.63 ± 0.66	0	3,750
S. sobrinus	5.0	4.27	0.73	117.27 ± 0.58	13.15 ± 0.12	0	1,352
	5.5	4.54	0.96	151.58 ± 0.15	29.97 ± 0.48	0	2,292
	6.0	4.81	1.19	111.52 ± 0.18	0	0	1,833
	7.0	6.22	0.78	97.19 ± 0.33	2.49 ± 0.22	0	2,611

Abbreviations as for Table 1.

Table 3 The pH, acid production and residual glucose at an initial glucose concentration of 5 mM, in oral streptococci

Species	pH_i	pH_f	ΔpH	Lactic acid (mM)	Acetic acid (mM)	G_f (mM)	Bacteria (mg/l)
S. gordonii	5.0	4.90	0.10	53.35 ± 0.22	22.98 ± 0.22	1.00 ± 0.01	842
	5.5	4.91	0.59	95.21 ± 0.25	25.64 ± 0.19	0	2,230
	6.0	5.46	0.54	67.97 ± 0.31	65.61 ± 0.25	0	2,750
	7.0	6.31	0.69	106.90 ± 0.35	34.97 ± 0.23	0	1,463
S. mutans	5.0	4.59	0.41	59.76 ± 0.22	0	3.10 ± 0.02	711
	5.5	4.77	0.73	76.83 ± 0.15	0	0	1,320
	6.0	4.36	1.64	186.37 ± 0.55	5.16 ± 0.13	0	2,115
	7.0	5.24	1.76	145.64 ± 0.51	0	0	2,531
S. oralis	5.0	NT	NT	NT	NT	NT	NT
	5.5	4.78	0.72	218.23 ± 0.55	38.96 ± 0.19	0	2,663
	6.0	4.40	1.60	175.43 ± 0.31	0.50 ± 0.09	0.62 ± 0.10	3,173
	7.0	5.81	1.19	66.74 ± 0.33	46.62 ± 0.45	0	4,583
S. salivarius	5.0	4.51	0.49	110.10 ± 0.54	14.48 ± 0.19	0.60 ± 0.20	1,130
	5.5	4.46	1.04	161.57 ± 0.66	10.65 ± 0.22	1.40 ± 0.20	2,578
	6.0	4.41	1.59	162.51 ± 0.64	13.82 ± 0.22	0	3,540
	7.0	5.23	1.77	141.21 ± 0.61	71.26 ± 0.55	1.82 ± 0.60	4,084
S. sanguis	5.0	4.92	0.08	29.41 ± 0.22	38.46 ± 0.51	0.30 ± 0.01	1,862
	5.5	4.56	0.94	191.46 ± 0.13	0	0	5,500
	6.0	4.76	1.24	104.64 ± 0.25	75.60 ± 0.23	2.50 ± 0.10	3,173
	7.0	5.84	1.16	123.49 ± 0.22	0	0	4,332
S. sobrinus	5.0	4.17	0.83	141.68 ± 0.55	10.32 ± 0.45	2.80 ± 0.30	1,100
	5.5	4.21	1.29	251.87 ± 0.44	9.99 ± 0.25	3.10 ± 0.60	1,919
	6.0	4.43	4.57	137.25 ± 0.53	0	0	2,578
	7.0	5.62	1.38	170.34 ± 0.43	19.15 ± 0.19	0	2,611

Abbreviations as for Table 1.

Table 4 The pH, acid production and residual glucose at an initial glucose concentration of 10 mM, in oral streptococci

Species	pH_i	pH_f	ΔpH	Lactic acid (mM)	Acetic acid (mM)	G_f (mM)	Bacteria (mg/l)
S. gordonii	5.0	4.78	0.22	75.50 ± 0.25	16.81 ± 0.15	2.02 ± 0.02	1,250
	5.5	4.64	0.86	98.03 ± 0.30	34.97 ± 0.16	0	1,571
	6.0	4.82	1.18	108.40 ± 0.22	57.28 ± 0.33	0.16 ± 0.02	3,587
	7.0	5.79	1.21	183.82 ± 0.55	17.65 ± 0.35	0.02 ± 0.01	3,113
S. mutans	5.0	4.61	0.39	62.03 ± 0.54	0	0	1,375
	5.5	4.33	1.17	167.89 ± 0.56	0.33 ± 0.10	0	1,905
	6.0	4.32	1.68	192.12 ± 0.46	10.32 ± 0.31	0	2,063
	7.0	4.80	2.20	179.48 ± 0.35	0	0.32 ± 0.10	2,714
S. oralis	5.0	NT	NT	NT	NT	NT	NT
	5.5	5.00	0.50	109.82 ± 0.15	6.16 ± 0.25	0	1,684
	6.0	4.27	1.83	204.09 ± 0.25	0	1.20 ± 0.02	3,056
	7.0	5.11	1.89	115.95 ± 0.64	0	0	771
S. salivarius	5.0	4.68	0.32	74.75 ± 0.13	10.49 ± 0.23	5.30 ± 0.30	938
	5.5	4.33	1.17	211.16 ± 0.15	0	0.60 ± 0.20	1,231
	6.0	4.11	1.89	110.57 ± 0.55	0	1.90 ± 0.30	3,173
	7.0	4.80	2.20	244.81 ± 0.67	82.42 ± 0.18	0	4,825
S. sanguis	5.0	5.03	0.03	24.32 ± 0.37	8.32 ± 0.32	2.32 ± 0.20	1,231
	5.5	4.73	0.77	124.53 ± 0.73	8.33 ± 0.17	0	4,583
	6.0	4.59	1.41	152.90 ± 0.42	38.30 ± 0.32	6.64 ± 0.20	3,750
	7.0	5.54	1.46	180.99 ± 0.15	0.67 ± 0.11	0	5,000
S. sobrinus	5.0	4.26	0.74	111.89 ± 0.55	6.49 ± 0.35	1.02 ± 0.30	959
	5.5	4.07	1.43	320.70 ± 0.44	0	0.80 ± 0.30	2,750
	6.0	1.15	1.85	166.19 ± 0.46	0	1.30 ± 0.20	2,661
	7.0	4.73	2.27	221.53 ± 0.34	0	0	3,327

Abbreviations as for Table 1.

In cultures of *S. sanguis*, the lowest final pH was 4.56, recorded from an initial value of 5.50. Acid production was always lowest at pH 5 but not always at pH 5.5; acid production was not concordant. Acetic acid production was maximal at pH 6, with large amounts of residual glucose.

The lowest pH attained in *S. sobrinus* at the end of the cultures was 4.07, recorded from an initial value of 5.50. Of all species tested, *S. sobrinus* produced the greatest net change in pH; *i.e.* a decrease from 7.00 in the initial culture to 4.73. At pH 5.5, lactic acid production approached 320.7 mM with 10 mM glucose.

Discussion

The decrease in plaque pH allows caries to appear, but the depth of the lesion is regulated by the concentration of non-ionized lactic acid outside the enamel, rather than by the pH itself (Featherstone *et al.*, 1979). It is nonetheless clear that plaque pH must fall below a critical value, and the amount of acid present must be large enough to dissolve calcium phosphates and allow the loss of minerals from the tooth to commence.

Micro-organisms which are able to grow well within the oral cavity as well as survive at acid pH value, and acidify the environment, are the ones most likely to cause caries (Loesche, 1986). Most oral bacteria are only able to develop within a narrow range of pH values between 6 and 8, the streptococci being a significant exception. However, not all streptococci are acidophilic. Bowden and Hamilton (1987) noted that at a pH of 5.5, *S. mitior* was unable to survive, whereas *S. mutans* survived well, and *S. sanguis* grew more slowly. When pH 4.5 was reached during culture, the number of *S. sanguis* and *S. mutans* cells decreased, although some cells continued to survive. Harper and Loesche (1984) reported that *S. sanguis* multiplied at pH 5.5, and reduced the pH in the culture to 4.7. DeSoet *et al.* (1989) recorded that this species did not develop at pH 5.

Although most oral streptococci are acidogenic (especially *S. sobrinus*) (DeSoet *et al.*, 1989), the rate of acid production varies from one species to another (Hamada and Slade, 1980). Among streptococcal species the type of acid produced has a fundamental effect on the appearance of caries. In most oral streptococci, the production of a specific type of acid depends on the available substrate; thus the formation of lactic acid is greater at high concentrations of glucose than at low concentrations. These differences are related to the inhibition of the enzyme pyruvate formate lyase (Marsh and Martin, 1984).

In the present study, we investigated the acidogenic, acidophilic and aciduric characteristics of *S. gordonii*, *S. mutans*, *S. oralis*, *S. salivarius*, *S. sanguis* and *S. sobrinus*. With the exception of *S. oralis*, which failed to grow at pH 5, all other oral streptococci continued to grow and produce acid at pH values between 5 and 7. In addition, the final pH found in cultures of each species depended to some extent on the initial pH: *S. gordonii*, *S. sanguis* and *S. sobrinus* caused the greatest decreases in pH from an initial value of 5.5, whereas in the other species, the largest decrease in pH was seen at a starting value of 6.0. The greatest decrease was that caused by *S. sobrinus*, confirming an earlier report by Hardie (1986). However, in the present study, *S. salivarius* brought about the second largest decrease in pH. These observations underline the importance of oral streptococci in caries formation, and show that the influence of *S. salivarius*, *S. gordonii* and *S. oralis* can be as important as that of *S. mutans*, *S. sobrinus* and *S. sanguis*.

The species we investigated produced acids mainly from glucose, although the amounts produced from peptides through degradative processes were not inconsiderable, because several amino acids (alanine, cysteine, glycine, serine, and threonine) in our degradation produced pyruvate, and the pyruvate can originate lactic and acetic acid by fermentation (Stryer, 1982). *S. salivarius* and *S. mutans* were particularly efficient at producing acids from peptides at certain pH values. In fact acid production from proteins surpassed production from the glucose substrate in these species. Under our conditions, more carbohydrates were apparently used to synthesize storage products than to produce acids. This factor should be taken into account in evaluations of the cariogenic potential of micro-organisms.

In general, at pH 6 and pH 7 the strains we investigated produced more acid at higher concentrations of glucose. At pH 5 and pH 5.5, increased glucose in the culture medium did not always enhance the production of lactic acid, or reduce that of acetic acid (DeSoet *et al.*, 1989). At low pH the ATPase, which controls transport mechanisms, may be blocked impeding the removal of intracellular protons and acids and leading to significant enzymatic alterations (Eisenberg and Marquis, 1981).

In conclusion, notable metabolic interactions take place in cariogenic dental plaque. Studies aimed at evaluating the cariogenic potential of oral streptococci traditionally considered to be of low cariogenic potential should take into account apparently conflicting data for lactic and acetic acid production with respect to residual glucose.

Acknowledgements

This work was partly supported by the Andalusian Regional Government through project 082. We thank Dr G. Piédrola and Dr M. C. Maroto of the Department of Microbiology, School of Medicine, University of Granada, for providing technical and administrative support. Our appreciation also goes to Dr M. L. Hayes of the Department of Biochemistry, University of Bristol, England, for help with the analytical techniques.

References

BOWDEN G. and Hamilton J. R. 1987. Environmental pH as a factor in the competition between strains of the oral streptococci *Streptococcus mutans, Streptococcus sanguis* and '*Streptococcus mitior*' growing in continuous culture. *Can. J. Microbiol.* **33** 824–7.

CARLSSON J., Iwami Y. and Yamada T. 1983. Hydrogen peroxide by oral streptococci and effect of lactoperoxidase-thiocyanate-hydrogen peroxidase. *Infect. Immun.* **40** 70–80.

DeSOET J. J., Toors F. A., and DeGraaf J. 1989. Acidogenesis by oral streptococci at different pH values. *Caries Res.* **23** 14–7.

DeSOET J. J., VanDalen P. J., Pavicic M. J. A. M. and DeGraaf J. 1990. Enumeration of *mutans* streptococci in clinical samples by using monoclonal antibodies. *J. clin. Microbiol.* **28** 2467–72.

EISENBERG A. and Marquis R. E. 1981. Enhanced transmembrane proton conductance in *Streptococcus mutans* GSS due to ionophoresis and fluoride. *Antimicrob. Agents Chemother.* **19** 807–12.

FACLAM R. R. 1977. Physiological differences of *viridans* streptococci. *J. clin. Microbiol.* **5** 185–201.

FEATHERSTONE J. D. N., Duncan J. F. and Cutress T. W. 1979. A mechanism for dental caries based on chemical processes and diffusion phenomena during *in vitro* caries simulation on human tooth enamel. *Arch. Oral Biol.* **24** 101–12.

GERHARDT P. (Editor) 1981. Divalent and biomass measurement. *In* Manual of Methods for General Biochemistry. pp 504–7. American Society of Microbiology, Washington, D.C., U.S.A.

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

HARDIE J. M. 1986. Oral streptococci. *In* Bergey's Manual of Systematic Bacteriology. Ninth editn. Vol 2, pp 1054–63. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe and J. G. Holt. Williams and Wilkins Co., Baltimore, U.S.A.

HARPER D. S. and Loesche W. J. 1984. Growth and acid tolerance of human dental plaque bacteria. *Arch. Oral Biol.* **29** 842–8.

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

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

MAIDEN M., Lai C. H-M. and Tanner A. 1992. Characteristics of oral Gram-positive bacteria. *In* Mosby Year Book. pp 342–72. Edited by J. Slots and M. Taubman. St. Louis, U.S.A.

MARSH P. and Martin M. (Editors). 1984. *In* Oral Microbiology. Second editn. pp 62–3. American Society of Microbiology. Washington, D.C., U.S.A.

SCHLEIFER K. H., Kilpper Balz R., Kraus J. and Gehring F. 1984. Relatedness and classification of *Streptococcus mutans* and '*mutans*-like' streptococci. *Dent. Res.* **63** 1047–50.

STRYER L. (Editor) 1982. Bioquímica. Second editn. Reverte SA, Madrid, Spain.

WHILEY R. A., Fraser H., Hardie J. M. and Beighton D. 1990. Phenotypic differentiation of *Streptococcus intermedius, Streptococcus constellatus* and *Streptococcus anginosus* strains within the '*Streptococcus milleri*' group. *J. clin. Microbiol.* **28** 1497–501.

Accepted 12 August 1996