ORIGINAL ARTICLE

Post-pH effect in oral streptococci

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Objective To study the post-pH effect (PpHE) in 11 strains of oral streptococci belonging to *Streptococcus sanguis*, *S. mitis*, *S. gordonii*, *S. mutans* and *S. sobrinus* by using the BacT/Alert microbial detection system.

Methods The bacterial cultures were exposed to a different pH (3.6, 4.0, 4.6, 5.0, 5.6, 7.0, 7.5, 8.0 and 8.6) for 1 h, and then returned to a neutral medium. The automated BacT/Alert system determined resumed microbial growth by measuring CO_2 generation. The PpHE expresses the time difference between resumed growth (after pH shock) and optimal growth (pH 7.0 used as the control).

Results PpHE was found to be minimal for alkaline pH values, and to be strain and species dependent.

Conclusions The PpHE may be useful as an ecological determinant or as an indicator of the cariogenicity of oral streptococci.

Key words Acidity, alkalinity, bacteria, cariogenicity, oral ecology

INTRODUCTION

Dental caries is a multifactorial process. Diet, the physical characteristics of the oral cavity, the pH of saliva and the types of microorganism present are all key determinants. Oral streptococci are the bacteria most frequently related to cariogenesis [1].

Some streptococci are able to produce acids (acidogenesis), grow in environments with an acidic pH (acidophilia), produce acids at low pH values (aciduric capacity) and synthesize intra- and extracellular polysaccharides [2]. Because these properties are not shared by all oral streptococci, cariogenic potential varies among species [3]. Several studies confirm a relationship between dental caries and *mutans* group streptococci, particularly *Streptococcus mutans* and *S. sobrinus* [4–10]. For this reason, it is important to accurately identify bacterial strains in accordance with current taxonomic standards [11–13].

Although the production of acid favors the appearance of caries due to demineralization, a lower pH may also inhibit further growth of some oral streptococci and other bacteria [5,14]. The pH exerts selective pressure on bacteria: micro-organisms that recover their normal rate of growth shortly after exposure to an acid pH have an ecological advantage over those that take longer to reinitiate growth. Thus, even temporary alterations of oral acidity or alkalinity (usually associated with changes in the amount of micronutrients available) may have considerable repercussions on bacterial growth [15].

The objective of our study was to determine the capacity of oral streptococci to resume growth after undergoing pH shock. This parameter, which we call the 'post-pH effect' (PpHE), was defined along the lines established by other authors for determining postantibiotic effect (with the Bactec system) [16] and post-H_2O_2 effect (with the BacT/Alert system) [17]. The PpHE is proposed as an in vitro ecological determinant to help assess the cariogenic potential of oral microflora.

MATERIALS AND METHODS

Microorganisms tested

The reference strains were *Streptococcus mitis* NCTC 11427, *S. gordonii* NCTC 3165, *S. sanguis* JENA 2697, *S. sanguis* NCTC 7863, and *S. mutans* NCTC 10449. Strains from the Oral Microbiology Laboratory of the School of Odontology, University of Granada were: *S. mitis* OGS 218, *S. mitis* OGS 232, *S. mitis* OGS 420, *S. mitis* OGS 628, *S. sobrinus* OGS 324, and *S. sobrinus* OGS 529. The OGS strains were isolated from dental plaque and saliva of different human subjects with dental caries,

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and were identified using the criteria of Maiden et al [18] and the method of Barrow and Feltham [19].

Post-pH effect

A bacterial suspension (turbidity 0.5 on the MacFarland scale) was prepared, with viable counts of 3×10^8. Then, 0.5 mL of the suspension was added to tubes containing 5 mL of trypticase soy broth without glucose (TSB; Scott, Madrid, Spain). The tubes were incubated for 1 h at $36 \pm 1°$C, after which 0.1 mL was transferred to tubes containing 3.0 mL of buffer at different pH values (3.6, 4.0, 4.6, 5.0, 5.6, 7.0, 7.5, 8.0 and 8.6). Buffers with pH 3.6–5.6 were obtained from a 0.2 M acetic acid and sodium acetate solution; those between pH 7.0 and 8.0 were from 0.2 M disodium hydrogen phosphate and sodium dihydrogen phosphate solution; and the pH 8.6 buffer was obtained from a 0.2 M solution of glycine and sodium hydroxide. The pH of each tube was confirmed after the addition of the inoculum.

The tubes of buffer plus inoculum were first incubated at $36 \pm 1°$C for 1 h. The bacteria were then washed twice with a buffer at pH 7.3 obtained from a 0.05 M solution of disodium hydrogen phosphate and sodium dihydrogen phosphate.

The pellet was resuspended in 3.5 mL of TSB, and 0.1 mL was taken to count viable cells.

Counts were done in Petri dishes containing Mitis Salivaris Agar (MSA; Difco Laboratories, Madrid, Spain), using dilutions of 10^{-1} to 10^{-6}. All assays were carried out in triplicate. Plates were incubated for 48 h at $36 \pm 1°$C in an anaerobic incubator, and counts were expressed as $\log_{10}$ CFU/mL.

The remaining 3.4 mL of bacterial culture was transferred to Organon Teknika bottles (no. 52296; Durham, North Carolina, USA), which were loaded into the incubator–reader of the BacT/Alert Microbial Detection System. The Bact/Alert system monitors the production of CO_2 to determine the presence of microorganisms [20].

The bottles were left in the system for a maximum of 60 h; the result was recorded as zero growth if the BacT/Alert system did not detect positive results during this time period. When microbial growth was detected, the cultures were reseeded on blood agar to check for contamination. The assays were done in triplicate for each strain at the different pH values.

The PpHE was defined as the difference between the time of detection of resumed growth by the BacT/Alert system after exposure for 1 h to a different pH (3.6, 4.0, 4.6, 5.0, 5.6, 7.5, 8.0 or 8.6) and the time of optimal growth as observed at a pH of 7.0.

Statistical analysis

We used the Kruskal–Wallis test and analysis of variance. All statistical analyses were done by the Department of Biostatistics and Operative Research of the University of Granada, using the Statigraphics version 5 software package (Statistical Graphics Corporation).

Results

Table 1 shows the mean cell counts in cultures at different pH values after incubation for 1 h at $36 \pm 1°$C. Four strains failed to grow at pH 3.6 (*S. sanguis* JENA 2697, *S. sanguis* NCTC 7863, *S. sobrinus* OGS 529 and *S. mitis* OGS 628). Very low counts were obtained at pH 3.6 for strains *S. gordonii* NCTC 3165, *S. mitis* OGS 420, and *S. mitis* OGS 218; as well as at pH 4 for strain *S. sanguis* JENA 2697. The highest counts were found at pH 7 for most strains.

Table 2 summarizes the time in hours needed by the BacT/Alert system to detect resumed growth in cultures exposed for

Table 1 Number of CFU/mL ($\log_{10}$) after acid or alkaline treatment with incubation for 1 h at $36 \pm 1°$C

Strains	pH								
	3.6	**4.0**	**4.6**	**5.0**	**5.6**	**7.0**	**7.5**	**8.0**	**8.6**
S. mitis NCTC 11427	4.00 ± 0.30^a	4.06 ± 0.20	4.14 ± 0.07	6.61 ± 0.04	6.84 ± 0.09	7.54 ± 0.04	7.49 ± 0.10	7.20 ± 0.04	7.19 ± 0.04
S. mitis OGS 232	4.20 ± 0.20	4.50 ± 0.50	5.80 ± 0.20	6.46 ± 0.10	7.67 ± 0.10	7.90 ± 0.10	7.09 ± 0.10	7.08 ± 0.09	7.09 ± 0.10
S. mitis OGS 420	2.81 ± 0.40	3.20 ± 0.08	3.80 ± 0.09	6.72 ± 0.25	8.85 ± 0.15	7.80 ± 0.10	7.50 ± 0.07	7.40 ± 0.36	7.13 ± 0.12
S. mitis OGS 218	2.84 ± 0.10	3.39 ± 0.10	6.17 ± 0.40	6.40 ± 0.06	6.38 ± 0.29	7.17 ± 0.10	6.82 ± 0.40	6.82 ± 0.02	6.75 ± 0.15
S. mitis OGS 628	–	3.22 ± 0.10	3.48 ± 0.06	5.84 ± 0.04	6.71 ± 0.019	7.90 ± 0.03	7.67 ± 0.20	7.67 ± 0.40	7.40 ± 0.29
S. sanguis NCTC 7863	–	3.24 ± 0.10	3.83 ± 0.10	4.24 ± 0.50	7.83 ± 0.18	7.80 ± 0.20	7.68 ± 0.10	7.16 ± 0.09	7.17 ± 0.14
S. sanguis JENA 2697	–	2.24 ± 0.10	3.46 ± 0.50	5.17 ± 0.30	7.57 ± 0.22	7.90 ± 0.10	7.90 ± 0.09	7.81 ± 0.20	7.70 ± 0.41
S. mutans NCTC 10449	4.82 ± 0.10	5.35 ± 0.05	6.83 ± 0.40	7.00 ± 0.08	6.41 ± 0.03	7.90 ± 0.20	7.80 ± 0.10	7.49 ± 0.09	7.46 ± 0.23
S. gordonii NCTC 3165	2.80 ± 0.10	2.85 ± 0.40	3.67 ± 0.40	6.69 ± 0.50	7.80 ± 0.04	7.90 ± 0.06	7.83 ± 0.20	7.65 ± 0.21	7.78 ± 0.45
S. sobrinus OGS 529	–	4.36 ± 0.4	4.40 ± 0.1	4.77 ± 0.3	6.38 ± 0.1	7.06 ± 0.2	6.74 ± 0.1	6.73 ± 0.9	6.72 ± 0.1
S. sobrinus OGS 324	3.43 ± 0.20	3.73 ± 0.40	3.90 ± 0.05	7.81 ± 0.10	7.00 ± 0.02	7.90 ± 0.30	7.90 ± 0.08	7.49 ± 0.09	7.70 ± 0.20

^{a} All values are the mean of three counts plus standard deviation.

Table 2 Delayed growth after acid or alkaline treatment expressed in hours, as detected by the BacT/Alert system

Microorganisms	pH								
	3.6	4.0	4.6	5.0	5.6	7.0	7.5	8.0	8.6
S. mitis NCTC 11427	14.32 ± 0.31	11.20 ± 0.35	9.46 ± 0.28	7.44 ± 0.22	5.31 ± 0.17	5.18 ± 0.19	5.21 ± 0.27	5.25 ± 0.26	5.47 ± 0.20
S. mitis OGS 232	15.17 ± 0.46	15.07 ± 0.42	8.24 ± 0.24	7.43 ± 0.20	6.49 ± 0.21	6.09 ± 0.21	6.21 ± 0.23	6.26 ± 0.24	6.37 ± 0.24
S. mitis OGS 420	24.51 ± 1.00	18.03 ± 0.55	14.15 ± 0.47	8.33 ± 0.33	7.31 ± 0.31	6.30 ± 0.13	7.03 ± 0.25	7.06 ± 0.28	7.17 ± 0.30
S. mitis OGS 218	47.03 ± 0.40	39.06 ± 0.58	25.11 ± 0.37	21.04 ± 0.36	7.41 ± 0.25	6.37 ± 0.26	6.40 ± 0.31	6.37 ± 0.36	6.56 ± 0.29
S. mitis OGS 628	—[a]	23.53 ± 0.34	16.54 ± 0.44	16.43 ± 0.39	12.31 ± 0.35	11.05 ± 0.24	11.04 ± 0.24	11.08 ± 0.27	11.23 ± 0.25
S. sanguis NCTC 7863	—	22.29 ± 0.55	21.02 ± 0.53	20.45 ± 0.36	6.40 ± 0.28	6.16 ± 0.35	6.59 ± 0.27	7.13 ± 0.29	8.35 ± 0.33
S. sanguis JENA 2697	—	20.39 ± 0.50	17.15 ± 0.54	14.21 ± 0.44	5.58 ± 0.24	5.55 ± 0.24	5.55 ± 0.30	6.05 ± 0.34	6.23 ± 0.28
S. mutans NCTC 10449	18.33 ± 0.49	16.06 ± 0.42	8.16 ± 0.34	8.16 ± 0.34	7.48 ± 0.26	6.46 ± 0.27	6.51 ± 0.28	7.29 ± 0.26	8.17 ± 0.33
S. gordonii NCTC 3165	22.22 ± 0.49	22.19 ± 0.48	21.41 ± 0.42	21.27 ± 0.55	7.58 ± 0	7.04 ± 0.32	7.10 ± 0.32	7.14 ± 0.32	7.18 ± 0.30
S. sobrinus OGS 529	—	15.00 ± 0.28	15.00 ± 0.20	14.42 ± 0.24	6.09 ± 0.21	5.29 ± 0.20	5.48 ± 0.14	6.02 ± 0.21	6.20 ± 0.22
S. sobrinus OGS 324	25.21 ± 0.52	25.12 ± 0.55	12.32 ± 0.45	12.01 ± 0.55	10.07 ± 0.34	9.31 ± 0.34	10.17 ± 0.35	11.59 ± 0.41	12.19 ± 0.48

[a] Indicates no growth.

Table 3 Post-pH effect (expressed in hours: compared with pH 7)

Microorganisms	pH							
	3.6	4.0	4.6	5.0	5.6	7.5	8.0	8.6
S. mitis NCTC 11427	9.14 ± 0.29	6.01 ± 0.32	4.27 ± 0.24	2.26 ± 0.23	0.12 ± 0.02	0.03 ± 0.09	0.06 ± 0.08	0.29 ± 0.01
S. mitis OGS 232	9.04 ± 0.24	8.59 ± 0.19	2.15 ± 0.06	1.33 ± 0.02	0.40 ± 0.03	0.12 ± 0.04	0.17 ± 0.03	0.28 ± 0.08
S. mitis OGS 420	18.41 ± 0.32	11.53 ± 0.26	8.05 ± 0.18	2.22 ± 0.05	1.21 ± 0.02	0.53 ± 0.04	0.56 ± 0.05	1.07 ± 0.01
S. mitis OGS 218	41.25 ± 0.55	32.29 ± 0.49	18.30 ± 0.25	14.47 ± 0.32	1.03 ± 0.19	0.19 ± 0.16	0.20 ± 0.23	0.18 ± 0.26
S. mitis OGS 628	—[a]	12.48 ± 0.41	5.40 ± 0.54	5.38 ± 0.48	1.26 ± 0.44	0.00 ± 0.02	0.03 ± 0.04	0.01 ± 0.03
S. sanguis NCTC 7863	—	16.43 ± 0.50	14.46 ± 0.41	14.39 ± 0.36	0.24 ± 0.07	0.43 ± 0.08	0.56 ± 0.06	2.29 ± 0.02
S. sanguis JENA 2697	—	14.55 ± 0.26	11.30 ± 0.30	8.36 ± 0.20	0.13 ± 0.01	0.10 ± 0.05	0.18 ± 0.09	0.38 ± 0.04
S. mutans NCTC 10449	11.46 ± 0.43	9.16 ± 1.02	1.29 ± 0.06	1.29 ± 0.07	1.01 ± 0.05	0.04 ± 0.03	0.42 ± 0.09	1.30 ± 0.41
S. gordonii NCTC 3165	15.00 ± 1.07	15.08 ± 0.51	14.37 ± 0.24	14.34 ± 0.24	0.54 ± 0.09	0.06 ± 0.03	0.10 ± 0.02	0.14 ± 0.03
S. sobrinus OGS 529	—	9.38 ± 0.47	9.31 ± 0.38	9.16 ± 0.42	0.40 ± 0.38	0.19 ± 0.19	0.35 ± 0.26	0.51 ± 0.26
S. sobrinus OGS 324	15.49 ± 0.43	15.41 ± 0.43	3.01 ± 0.47	2.30 ± 0.21	0.36 ± 0.04	0.46 ± 0.03	2.28 ± 0.06	2.47 ± 0.16

[a] Indicates no growth.

1 h to different pH values. The results varied widely, the shortest times corresponding to pH 7. The strains that had failed to grow at pH 3.6 continued to read negative. For some strains, the system took over 24 h to detect resumed growth (*S. mitis* OGS 420 at pH 3.6, *S. sobrinus* OGS 324 at pH 3.6 and pH 4.0, and *S. mitis* OGS 218 at pH 3.6, 4.0 and 4.6).

Table 3 gives the PpHE for each strain and pH. A low PpHE value means that the strain resumed growth shortly after pH shock, whereas a high PpHE means that growth was delayed.

The Kruskal–Wallis test revealed significant differences in the PpHE of the 11 strains assayed. We found a significant association between PpHE and pH, and between PpHE and strain. Analysis of variance with strain as the factor of variation confirmed that PpHE was strain-dependent ($P \leqslant 0.0001$).

The results of the two-by-two contrast were inconsistent, with differences in PpHE appearing in some cases but not in others. For example, at pH 3.6 there was no significant difference between strains *S. mitis* NCTC 11427 and *S. mitis* OGS 232, and at pH 4.0 there was no difference between *S. sobrinus* OGS 529 and *S. mutans* NCTC 10449. Considerable variation in PpHE was found at pH 4.0 and 4.6, whereas the values were lower and much more homogeneous at pH 5.6. The PpHE increased slightly from pH 7.5 through pH 8.6, although the values remained relatively low and homogeneous.

DISCUSSION

Most oral bacteria can sustain optimal growth only within a narrow range of pH values from 6 to 8. The oral streptococci able to survive at a low pH and to continue producing acid under these conditions are thought to be the most cariogenic [4]. With some exceptions, the ability to grow at pH 5.5 is considered to mark the difference between *mutans* and non-*mutans* acidophilic streptococci [5,21]. The rate of acid production and the aciduric capacity also vary among species [22]; both properties are thought to be strain dependent [7].

The pH of the oral cavity is therefore an important ecological determinant. Yet the complex synergistic and antagonistic processes of the oral cavity are poorly understood, in part because they cannot be accurately reproduced in vitro. For example, sudden variations in pH in the oral cavity that affect the growth of microorganisms may go undetected, as they do not always cause demineralization or the formation of calculus [15].

Microorganisms that recover quickly from fluctuations in pH will be better able to colonize and produce plaque, thus becoming pathogens. Slow recovery after pH shock means that pathogenicity is reduced, or at least delayed. The post-pH effect, as defined in this study, would indicate the capacity of oral streptococci—the most abundant microbes in supragingival plaque—to recover from pH shock and resume growth.

Our results show that viable cell counts after acidic pH shock are heterogeneous. Although acidity (especially lower than pH 5.0) caused a notable decrease in the numbers of viable cells, not all species or strains of a given species behaved in the same way: some strains of *S. mitis* failed to grow after exposure to pH 3.6, as did both strains of *S. sanguis* tested. At alkaline pH values, the CFU per milliliter counts were similar for all the strains tested. In contrast, even a mildly acidic pH resulted in noteworthy variations of bacterial cell viability.

In those cases where viable cell count was lower than the count for the pH 7 control, we had anticipated that the BacT/Alert system would take longer to detect resumed growth. However, this premise did not hold in all cases. This finding may be attributed to the varying degrees of metabolic activity in cells that remain viable. If acid pH values affect strains of oral streptococci differently, the time needed for the bacteria to recover would differ as well [17].

It is therefore not surprising that the PpHE was found to vary considerably among the oral streptococci tested. In general, the time of PpHE was reduced for alkaline pH, and longer for acid pH values (especially pH 3.6). A pH of 5.6 appears to be the cut-off point above which PpHE is reduced. In other words, oral streptococci, in vitro at least, are more resistant to alkaline environments. Therefore, when intraplaque pH increases, as can occur during periods of food deprivation, these bacteria may continue to develop at a nearly normal rate. In contrast, if the pH drops abruptly, their development is severely restricted as long as the environment remains acidic.

De Soet et al [7] found viable cell count after pH shock to be species and strain dependent. Our results suggest that viable cell count after pH shock, the time needed to detect positive bacterial growth using the BacT/Alert system, and the corresponding PpHE value, are all species and strain dependent. Nonetheless, the short PpHE times were not always observed in strains usually considered to be the most cariogenic; likewise, the PpHE is not necessarily longer for strains considered less cariogenic.

In conclusion, the PpHE may prove to be useful as an indicator of environmental conditions in the oral cavity, and as a determinant of cariogenicity among oral streptococci.

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