Metabolic cooperation in oral microbial communities during growth on mucin

D. J. Bradshaw,¹ K. A. Homer,² P. D. Marsh¹ and D. Beighton²

Author for correspondence: D. Beighton. Tel: +44 71 346 3608. Fax: +44 71 346 3624.

¹ Research Division, Centre for Applied Microbiology and Research (CAMR), Porton Down, Salisbury SP4 0JG, UK

² Oral Microbiology, Royal College of Surgeons Department of Dental Sciences, King's College School of Medicine and Dentistry, Faculty of Clinical Dentistry, Caldecot Road, Denmark Hill, London SE5 9RW, UK

Hog gastric mucin has been used as a model glycoprotein to determine the role of particular glycosidases produced by different oral bacteria in the development of stable, diverse microbial communities. The patterns of glycosidase and protease activity were determined in pure cultures of ten representative species of oral bacteria using synthetic substrates. A five-member mixed culture was established in a chemostat, comprising species with minimal glycosidase and protease activity, in which hog gastric mucin was the major carbon and energy source. Introduction of additional species with novel enzyme activities (e.g. sialidase, α -fucosidase and endopeptidase) led to their establishment within the community to make communities with seven, eight, nine and ten members and resulted in an increase in the total viable counts of the microbial consortium. This increase in viable count was made up of the numbers of the newly added species as well as from a rise in the numbers of the existing community members. This result suggested that glycoprotein catabolism involved the synergistic and concerted action of several species with overlapping patterns of enzyme activity. Such metabolic cooperation results in the liberation of additional nutrients, and this may help to maintain the characteristic diversity of resident microbial communities found in many natural habitats.

Keywords: dental plaque, bacterial communities, chemostat, glycosidase, mucin

INTRODUCTION

The growth of bacteria in the oral cavity involves the degradation of host-derived glycoproteins (including salivary mucins) and proteins (Beckers & van der Hoeven, 1982; Beighton & Hayday, 1986; Beighton *et al.*, 1986). The particular glycosidic enzymes produced include sialidase, β -galactosidase, β -N-acetylglucosaminidase, β -N-acetylgalactosaminidase, α - and β -mannosidase and α -fucosidase (Kilian *et al.*, 1989; Beighton *et al.*, 1991; Slots, 1981). These enzymes can catalyse hydrolysis of the oligosaccharide sidechains and the liberation of carbohydrates which are thus made available to the resident microflora for growth. Additionally, removal of the oligosaccharide sidechains increases the proteolytic susceptibility of the protein core (Gottschalk & Fazekas de St Groth, 1960; Sharon & Lis, 1982), which can thus be degraded by a wide range of bacterial exo- and endo-proteolytic enzyme activities to release peptides and amino acids which may then be utilized. The effect of these

bacterial activities is that the growth rate of bacteria in dental plaque can be sustained even in the absence of host diet (Beckers & van der Hoeven, 1982; Beighton & Hayday, 1986; Beighton *et al.*, 1986). Withdrawal of the host diet leads to significant increases in the plaque levels of these specific glycosidic and non-specific proteolytic enzyme activities (Smith & Beighton, 1986, 1987).

In order to study the role of the resident microflora in the catabolism of complex, endogenous molecules, hog gastric mucin (HGM) (Beighton *et al.*, 1988; Glenister *et al.*, 1988; Bradshaw *et al.*, 1989) or heat-inactivated human serum (ter Steeg *et al.*, 1988) have been used as model substrates. When pooled dental plaque was used as the inoculum in continuous culture studies these substrates were found to support complex microbial communities of plaque bacteria (Beighton *et al.*, 1988; Glenister *et al.*, 1988; Bradshaw *et al.*, 1989; ter Steeg *et al.*, 1988). In such studies, bacterial growth was accompanied by the production of degradative enzyme activities and by the utilization of the added glycoproteins. However, the use of complex, poorly defined inocula did not readily enable the role of particular species to be established. Batch

Abbreviations: HGM, hog gastric mucin; 4-MU, 4-methylumbelliferyl; AMC, 7-amido-4-methylcoumarin.

culture studies have demonstrated that simple combinations of certain species of dental plaque bacteria degrade proteins and glycoproteins synergistically (Gharbia *et al.*, 1989; van der Hoeven & Camp, 1991; Homer & Beighton, 1992).

The aim of the present study, therefore, was to investigate further the role of particular enzymes produced by members of the resident oral flora in (i) the persistence of individual species, and (ii) the development and stability of oral microbial communities, when growing on complex macromolecules. These studies were performed by establishing increasingly diverse (but defined) mixed cultures of oral bacteria in a chemostat in which HGM was the main carbon and energy source. HGM was used as the model substrate because it shows the highest similarity in oligosaccharide structure with human salivary mucin (Herp *et al.*, 1979).

METHODS

Bacterial strains. The bacteria used in this study were *Streptococcus mutans* R9, *Streptococcus oralis* EF186, *Streptococcus gordonii* NCTC 7865, *Neisseria subflava* A1078, *Veillonella dispar* ATCC 17745, *Lactobacillus casei* AC413, *Actinomyces naeslundii* (formerly *Actinomyces viscosus*, Johnson *et al.*, 1990) WVU 627, *Porphyromonas gingivalis* W50, *Prevotella nigrescens* T588 (formerly *P. intermedia*, Shah & Gharbia, 1992) and *Fusobacterium nucleatum* ATCC 10953. These species were chosen on the basis of their relevance in oral microbial ecology, and for their ease of identification (McKee *et al.*, 1985). They were maintained and stored as previously described (Bradshaw *et al.*, 1989).

Growth of bacteria in batch culture. The production of glycosidases by the above bacteria was determined by culturing each strain, in duplicate batch culture, in complex medium (modified BM; McKee *et al.*, 1985) diluted 1:5 (BM/5) but supplemented with 0.25% HGM (Sigma). The final composition of the medium was thus (g per l distilled water): HGM, 2.5; proteose peptone (Oxoid), 2; yeast extract (Difco), 1; cysteine.HCl (Sigma), 0.1; haemin (Sigma), 0.001. The pH was adjusted to 7.4 prior to autoclaving. When bacteria were grown in pure culture in BM/5 without HGM, growth was insufficient for enzyme analysis. After appropriate incubation (3 d aerobically for *N. subflava*, 4 d anaerobically for *L. casei*, *A. naeslundii* and all the streptococcal species, and 6 d anaerobically for *Por. gingivalis*, *Pre. nigrescens*, *F. nucleatum* and *V. dispar*), cells were harvested by centrifugation. Supernatants were decanted and stored at -20 °C, as were the bacterial pellets. All cell pellets and culture supernatants were assayed for specific glycosidic and proteolytic enzyme activities.

Measurement of bacterial enzyme activities. Glycosidic and proteolytic enzyme activities were measured using fluorogenic substrates essentially as previously described (Homer *et al.*, 1992). Briefly, specific glycosidase activities were determined using 4-MU-linked substrates (4-MU- α -L-fucoside, 4-MU- α -N-acetylneuraminic acid, 4-MU-N-acetyl- β -D-glucosaminide, 4-MU- β -D-mannoside, 4-MU- β -D-galactoside, 4-MU-N-acetyl- β -D-galactosaminide) while proteolytic activities were monitored using glycyl-prolyl-AMC and benzoyl-arginyl-AMC as model substrates. Bacterial cell pellets were thawed at ambient temperature and resuspended in 50 mM TES buffer, pH 7.5. Enzyme assays were set up in clear, flat-bottomed microtitre trays, using 50 μ l substrate solutions (100 μ g ml⁻¹) in TES buffer, 50 μ l TES buffer and 100 μ l cell suspension or culture

supernatant. The TES buffer was supplemented with 4 mM dithiothreitol for assays with the anaerobic bacteria. All assays were performed in duplicate and incubated anaerobically at 37 °C. Control assays contained TES buffer in place of bacterial suspensions or culture supernatants. Relative fluorescence values were read at intervals over 5 h on a fluorimeter fitted with a plate reading attachment (LS-3B, Perkin-Elmer) at excitation and emission wavelengths of 380 nm and 460 nm, respectively. Product formation was estimated by comparison of fluorescence values with standard curves of either 4-methylumbelliferone or 7-amido-4-methylcoumarin. The protein concentration of bacterial cell suspensions was estimated after extraction with 0.5 M NaOH with the Coomassie Blue dye-binding assay (Sigma) using bovine serum albumin (Sigma) as the standard. Specific enzyme activities were expressed as nmol h⁻¹ (mg protein)⁻¹ or as nmol h⁻¹ (ml supernatant)⁻¹.

Chemostat growth of mixed bacterial communities. Increasingly diverse bacterial communities were grown in a chemostat as described previously (Bradshaw *et al.*, 1989). The chemostat growth medium was BM/5 supplemented with 0.25% HGM; the pH and temperature were maintained automatically at 7.0 $\pm$ 0.1 and 37 °C, respectively, and the dilution rate (*D*) was 0.1 h⁻¹ (equivalent to a mean generation time of 6.9 h). The working volume was 500 ml, and the gas phase was 5% CO₂ in N₂ (Distillers).

Initially a five-species community was established in the chemostat by inoculating the culture with a pooled inoculum consisting of *S. mutans* R9, *S. gordonii* NCTC 7865, *N. subflava* A1078, *V. dispar* ATCC 17745, and *F. nucleatum* ATCC 10953. These organisms were selected as they constitute commonly isolated members of the normal plaque microflora but which, with the exception of *S. gordonii*, do not produce detectable glycosidase activities relevant to the degradation of the oligosaccharide side chains of HGM. A steady state was allowed to establish (5 d at *D* = 0.1 h⁻¹) and culture samples were taken for microbiological analysis.

In order to introduce sialidase activity, *S. oralis* EF186 and *A. naeslundii* WVU 627 were inoculated into the five-species community to establish a seven-species culture. A new steady state was established and samples were removed for microbiological analysis. An eight-species community was established with the addition of *L. casei* AC413, which had α -fucosidase activity, and appropriate culture samples were again taken. The complexity of the community was then further increased by the addition of *Por. gingivalis* W50 (with high endopeptidase activity and the ability to degrade benzoyl-arginyl-AMC) to form a nine-species community. The most diverse community, a ten-species community, was achieved by the addition of *Pre. nigrescens* T588 (with moderate endopeptidase activity as judged by glycyl-prolyl-AMC hydrolysis and unable to hydrolyse benzoyl-arginyl-AMC). A new steady state was achieved before samples were taken for viable counts.

Enumeration of bacteria in chemostat samples. Individual bacterial species in samples taken from the chemostat were enumerated by decimal dilution of the culture and plating aliquots on a range of selective and non-selective culture media as described previously (Bradshaw *et al.*, 1989). Multiple samples were taken during each steady state. The detection limits for the species were 10 c.f.u. ml⁻¹ for *A. naeslundii*, *N. subflava* and *L. casei*; 10² c.f.u. ml⁻¹ for *Pre. nigrescens*, *S. gordonii* and *S. mutans*; 10³ c.f.u. ml⁻¹ for *F. nucleatum*; 10⁴ c.f.u. ml⁻¹ for *V. dispar* and *S. oralis*; and 10⁵ c.f.u. ml⁻¹ for *Por. gingivalis*. The total number of bacteria in each steady-state community and the individual bacterial counts were expressed as log₁₀(c.f.u. ml⁻¹), and the mean $\pm$ standard deviation (SD) of individual counts for each

Table 1. Production of selected glycosidases and proteases by individual bacteria grown in batch culture (BM/5 + mucin medium)

Species	Enzyme activity [nmol substrate hydrolysed h ⁻¹ (mg protein) ⁻¹]					
	Sialidase	N-Acetyl-glucosaminidase	β -Galactosidase	α -Fucosidase	Gly-Pro-diamino peptidase	Trypsin-like activity*
<i>S. mutans</i>	ND	ND	ND	ND	747	ND
<i>S. gordonii</i>	ND	566	36	50	11 000	0.7
<i>N. subflava</i>	ND	ND	ND	ND	ND	ND
<i>V. dispar</i>	ND	2	ND	ND	ND	ND
<i>F. nucleatum</i>	ND	33	ND	ND	ND	ND
<i>S. oralis</i>	10 900	300	ND	ND	13 900	ND
<i>A. naeslundii</i>	260	ND	17	ND	ND	ND
<i>L. casei</i>	ND	14	ND	2453	495	ND
<i>Por. gingivalis</i>	8	ND	ND	ND	216	1552
<i>Pre. nigrescens</i>	ND	20	ND	507	405	ND

ND, Not detected (insignificant enzyme activity).

* Trypsin-like activity measured by hydrolysis of benzoyl-Arg-AMC substrate.

Table 2. Growth of various defined mixed communities of oral bacteria on mucin-supplemented medium

Species	Mean log ₁₀ (c.f.u. ml ⁻¹) $\pm$ SD (n)				
	Mixed 5	Mixed 7	Mixed 8	Mixed 9	Mixed 10
<i>S. mutans</i>	4.03 $\pm$ 0.51 (5)	4.61 $\pm$ 0.23 (5)	4.55 $\pm$ 0.57 (4)	4.93 $\pm$ 0.13 (3)	4.84 $\pm$ 0.08 (2)
<i>S. gordonii</i>	7.32 $\pm$ 0.20 (5)	7.54 $\pm$ 0.12 (5)	7.80 $\pm$ 0.05 (4)	7.93 $\pm$ 0.09 (3)	7.91 $\pm$ 0.03 (2)
<i>N. subflava</i>	3.50 $\pm$ 0.16 (5)	4.73 $\pm$ 0.40 (5)	5.60 $\pm$ 0.29 (4)	5.73 $\pm$ 0.23 (3)	5.82 $\pm$ 1.10 (2)
<i>V. dispar</i>	5.94 $\pm$ 0.61 (5)	6.61 $\pm$ 0.28 (5)	7.43 $\pm$ 0.10 (4)	7.59 $\pm$ 0.06 (3)	7.46 $\pm$ 0.17 (2)
<i>F. nucleatum</i>	7.75 $\pm$ 0.12 (5)	7.74 $\pm$ 0.28 (5)	7.52 $\pm$ 0.15 (4)	7.77 $\pm$ 0.13 (3)	7.56 $\pm$ 0.67 (2)
<i>S. oralis</i>	—	6.38 $\pm$ 0.62 (5)	7.00 $\pm$ 0.20 (4)	6.85 $\pm$ 0.33 (3)	7.10 $\pm$ 0.08 (2)
<i>A. naeslundii</i>	—	5.98 $\pm$ 0.33 (5)	5.27 $\pm$ 0.48 (4)	5.10 $\pm$ 0.17 (3)	4.78 $\pm$ 0.09 (2)
<i>L. casei</i>	—	—	5.23 $\pm$ 0.15 (4)	5.60 $\pm$ 0.30 (3)	5.89 $\pm$ 0.41 (2)
<i>Por. gingivalis</i>	—	—	—	7.72 $\pm$ 0.06 (3)	7.63 $\pm$ 0.28 (2)
<i>Pre. nigrescens</i>	—	—	—	—	7.02 $\pm$ 0.03 (2)
Total	7.91 $\pm$ 0.09 (5)	8.02 $\pm$ 0.15 (5)	8.13 $\pm$ 0.64 (4)	8.39 $\pm$ 0.05 (3)	8.37 $\pm$ 0.20 (2)

steady-state community were calculated. Mean values were compared initially by ANOVA, and then by pair-wise comparisons of the steady-state communities, which were made using the Scheffe multiple comparison test (Statgraphics) with $P < 0.05$ accepted as the level of significance.

RESULTS

Enzyme activities of individual species in batch culture

The enzyme activities of each of the species grown in pure culture are summarized in Table 1. Individual species

varied markedly in their range of glycosidic and endopeptidase activities. No single species produced all of the enzymes assayed for.

Consideration of these data enabled defined mixed cultures to be constructed with precise patterns of glycosidase and protease activities. Thus, the bacterial species selected for the initial bacterial community possessed minimal β -galactosidase and α -fucosidase activity and no sialidase activity, thereby limiting its ability to catabolize HGM (Table 1). The addition of *S. oralis* and *A. naeslundii* to form a seven-species community would result in the

Fig. 1. Total viable count of microbial communities as their diversity is increased. The figure shows the numerical contribution of the added species and their effect on the levels of the extant bacterial species. ■, Extant (5, 7, 8, 9); ▨, increase in extant; ▩, newly added.

introduction of organisms with a marked capacity to produce sialidase. The addition of *L. casei* to form an eight-species community would markedly increase the production of α -fucosidase activity, while the introduction of *Por. gingivalis* to form a nine-species community substantially increased the total endopeptidase activity of the community. The inoculation of *Pre. nigrescens* would not introduce any novel enzyme activities as determined from this range of substrates.

Bacterial population dynamics of defined mixed cultures

The five-species community reached a steady state in the chemostat. Stable steady-state communities were also attained following the addition of species to make mixed cultures with seven, eight, nine and ten members (Table 2). The total viable count of the steady-state mixed cultures increased significantly ($P < 0.001$) from 7.91 ± 0.05 in the five-species community to 8.40 ± 0.07 in the nine-species community; there was a small, non-significant reduction to 8.37 ± 0.08 in the ten-species community (Table 2). Fig. 1 shows that the increase in the numbers of bacteria is a combination of an increase in the numbers of bacteria in the extant community plus a contribution from the newly added species.

The overall effect on the levels of individual species as the diversity of the community increased is shown in Table 2. Viable counts of *N. subflava* and *V. dispar* increased markedly, particularly when the diversity of the community increased from five to eight species. The levels of other species such as *S. mutans*, *S. gordonii* and *L. casei* also rose, but to a lesser degree. In contrast, the mean viable counts of *F. nucleatum* and *S. oralis* remained relatively constant within microbial communities of increasing diversity; this was also observed with *Por. gingivalis*.

DISCUSSION

The growth of bacteria in the oral cavity is primarily dependent upon the ability of dental plaque bacteria to utilize endogenous nutrients, especially glycoproteins, including salivary mucins. Observations in rats (Beckers & van der Hoeven, 1982) and macaque monkeys (Beighton & Hayday, 1986; Beighton *et al.*, 1986) have shown that withdrawal of the diet, but not drinking water, for short periods does not significantly alter the rate of bacterial growth in dental plaque. Likewise, fasted laboratory animals (Kilian & Rolla, 1976) accumulate dental plaque with a similar microbial composition to that formed during conventional feeding. Bacteria may liberate nutrients for growth from the oligosaccharide side-chains of glycoproteins, from peptides derived from the protein core of glycoproteins, and from non-glycosylated proteins, by the action of specific glycosidases and non-specific proteases, respectively. Changes in the levels of these enzyme activities can be measured in dental plaque of monkeys receiving different dietary regimens (Beighton & Smith, 1986; Smith & Beighton, 1986, 1987).

In laboratory studies using simple and defined mixtures of bacteria, combinations of *S. mutans* and *Streptococcus sobrinus* with either, or both, *A. naeslundii* and *F. nucleatum* degraded human transferrin and bovine serum albumin synergistically (Homer & Beighton, 1992) while *Por. gingivalis* in combination with *F. nucleatum* hydrolysed casein synergistically (Gharbia *et al.*, 1989) and mixtures of *S. oralis* and *S. sanguis* degraded HGM synergistically (van der Hoeven & Camp, 1991). Chemostat studies using complex microbial communities derived from pooled dental plaque have indicated that the degradation of serum proteins is accomplished by the synergistic and concerted action of various bacteria; different stable bacterial populations were also found to exhibit the same metabolic capabilities (ter Steeg *et al.*, 1988).

In the present study, HGM has been used as a model glycoprotein as its oligosaccharide structure is similar to that of many human glycoproteins (Herp *et al.*, 1979). The carbohydrates in the side chains of mucin-type (O-linked) glycoproteins are primarily sialic acid, galactose, *N*-acetylglucosamine and fucose and are linked via either serine or threonine to the protein core. These carbohydrates are present in different combinations, though sialic acid and fucose are always terminal and galactose and *N*-acetylglucosamine are β -linked. Mannose, present in *N*-linked oligosaccharide side-chains, may be either α - or β -linked (Herp *et al.*, 1979). The diversity of these side-chains and of the protein core result in a complex molecule whose degradation will result only from the action of several bacterial enzymes. The actions of the enzymes produced by individual species results in the liberation of nutrients from different regions of the macromolecule. Perhaps such large molecules may be considered to possess an array of discrete 'nutrient niches', each of which may be exploited by a limited number of species. A consortium of bacteria is necessary, therefore, to maximize the nutritional exploitation of such a complex substrate, as has been proposed for the human colon (Hoskins *et al.*,

1985). Sugars released during the breakdown of these glycoproteins can support the growth of non-mucin-degrading species (Hoskins *et al.*, 1992).

In the present study, a five-species community was able to establish in the chemostat and reach a steady state. Subsequently, further steady states were achieved when additional species were introduced. Implicit in this observation is that microbial interactions are occurring (Veldkamp, 1976), and/or the growth of the component species was not being limited by the same nutrient (Alexander, 1971; Pirt, 1975). Further, in each steady-state community a given species might be limited by a different nutrient. The total number of bacteria (c.f.u. ml⁻¹) rose significantly as the species diversity increased, especially from the five-species community to the nine-species community. However, there was no significant increase in bacterial numbers upon the addition of *Pre. nigrescens* to the nine-species community, confirming that the introduction of this species was not accompanied by the addition of any novel degradative abilities. The order in which the bacteria were introduced may influence the steady-state community structures, as we were unable to introduce successfully *Pre. nigrescens* into the five-species community (data not shown).

The rise in viable count of the community as the species diversity was increased was not due simply to the numbers of added cells. Generally, there was a greater contribution due to the increase in numbers of some existing members of the community. Thus, the introduction of further degradative enzyme activities not only enabled the newly added species to establish within the existing microbial community but, in doing so, also provided additional substrates for growth and further catabolic opportunities for species within the pre-existing populations.

There appeared to be three types of microbial response as the diversity of the community increased step-wise. Firstly, there was an increase in the numbers of the pre-existing members of the community for the reasons described above (although *V. dispar* may also increase in numbers due to the elevated levels of lactate arising from the metabolism of the HGM-derived carbohydrates by other species). Secondly, there were certain species, especially *F. nucleatum*, which remained at a constant level throughout the changes to the species diversity of the community. This implies that the metabolism of these species was not markedly influenced by other organisms. This may be because they neither co-operate nor compete with other members of this model community, i.e. their metabolic niche or domain does not coincide or overlap appreciably with those of the other species in the present consortium. This is supported by some observations of cultures grown in the absence of HGM, in which *F. nucleatum* predominated, indicating that it depended largely upon the peptide components of the medium for its success in the culture (unpublished observations). Thirdly, the consistent loss of *A. naeslundii* with each step-wise addition of other species implied that competition could occur between the new and existing members of the community. The observation that *A. naeslundii* was not totally displaced from the community underlines the

diversity of nutritional niches that are presented to bacteria by macromolecules as complex as glycoproteins.

In conclusion, this study has confirmed that HGM can support the growth of diverse bacterial communities. The degradation of glycoproteins involves the synergistic and concerted action of several species which possess complementary patterns of glycosidase and protease activities. The metabolism of such molecules is one mechanism by which the stability and diversity of microbial communities, characteristic of many natural environments, may be maintained.

Alexander, M. (1971). Nutrition. In *Microbial Ecology*, pp. 94–125. New York: Wiley.

Beckers, H. J. A. & van der Hoeven, J. S. (1982). Growth rates of *Actinomyces viscosus* and *Streptococcus mutans* during early colonization of tooth surfaces in gnotobiotic rats. *Infect Immun* **35**, 583–587.

Beighton, D. & Hayday, H. (1986). The influence of diet on the growth of streptococcal bacteria on the molar teeth of monkeys (*Macaca fascicularis*). *Arch Oral Biol* **31**, 449–454.

Beighton, D. & Smith, K. (1986). The modulation of exoglycosidic enzymes in the supragingival plaque of macaque monkeys. *FEMS Microbiol Lett* **34**, 319–322.

Beighton, D., Smith, K. & Hayday, H. (1986). The growth of bacteria and the production of exoglycosidic enzymes in the dental plaque of macaque monkeys. *Arch Oral Biol* **31**, 829–835.

Beighton, D., Smith, K., Glenister, D. A., Salamon, K. E. & Keevil, C. W. (1988). Increased degradative enzyme production by complex communities of dental plaque bacteria in mucin-limited continuous culture. *Microb Ecol Health Dis* **1**, 85–94.

Beighton, D., Hardie, J. M. & Whaley, R. A. (1991). A scheme for the identification of viridans streptococci. *J Med Microbiol* **35**, 367–372.

Bradshaw, D. J., McKee, A. S. & Marsh, P. D. (1989). Effects of carbohydrate pulses and pH on population shifts within oral microbial communities in vitro. *J Dent Res* **68**, 1298–1302.

Gharbia, S. E., Shah, H. N. & Welch, S. G. (1989). The influence of peptides on the uptake of amino acids in *Fusobacterium*; predicted interactions with *Porphyromonas gingivalis*. *Curr Microbiol* **19**, 231–235.

Glenister, D. A., Salamon, K. E., Smith, K., Beighton, D. & Keevil, C. W. (1988). Enhanced growth of complex communities of dental plaque bacteria in mucin-limited continuous culture. *Microb Ecol Health Dis* **1**, 31–38.

Gottschalk, A. & Fazekas de St Groth, S. (1960). Studies on mucoproteins III. The accessibility to trypsin of the susceptible bonds in ovine submaxillary gland mucoprotein. *Biochem Biophys Acta* **43**, 513–519.

Herp, A., Wu, A. M. & Moschera, J. (1979). Current concepts of the structure and nature of mammalian salivary mucous glycoproteins. *Mol Cell Biochem* **23**, 27–44.

Homer, K. A. & Beighton, D. (1992). Synergistic degradation of transferrin by mutans streptococci in association with other dental plaque bacteria. *Microb Ecol Health Dis* **5**, 111–116.

Homer, K. A., Manji, F. & Beighton, D. (1992). Inhibition of peptidase and glycosidase activities of *Porphyromonas (Bacteroides) gingivalis*, *Bacteroides intermedius* and *Treponema denticola* by plant extracts. *J Clin Periodontol* **19**, 305–310.

Hoskins, L. C., Agustines, M., McKee, W. B., Boulding, E. T.,

- Kriaris, M. & Niedermeyer, G. (1985). Mucin degradation in human colon ecosystems. Isolation and properties of fecal strains that degrade ABH blood group antigens and oligosaccharides from mucin glycoproteins. *J Clin Invest* **75**, 944–953.
- Hoskins, L. C., Boulding, E. T., Gerker, T. A., Harouny, V. R. & Kriaris, M. S. (1992). Mucin glycoprotein degradation by mucin oligosaccharide-degrading strains of human faecal bacteria. Characterisation of saccharide cleavage products and their potential role in the nutritional support of larger faecal populations. *Microb Ecol Health Dis* **5**, 193–207.
- Johnson, J. L., Moore, L. V. H., Kaneko, B. & Moore, W. E. C. (1990). *Actinomyces georgiae* sp. nov., *Actinomyces gerencseriae* sp. nov., designation of two genospecies of *Actinomyces naeslundii*, and inclusion of *A. naeslundii* serotypes II and III and *Actinomyces viscosus* serotype II in *A. naeslundii* genospecies 2. *Int J Syst Bacteriol* **40**, 273–286.
- Kilian, M. & Rölla, G. (1976). Initial colonization of teeth in monkeys as related to diet. *Infect Immun* **14**, 1022–1027.
- Kilian, M., Mikkelsen, L. & Henriksen, J. (1989). Taxonomic study of viridans streptococci: description of *Streptococcus gordonii* sp. nov. and amended descriptions of *Streptococcus sanguis* (White and Niven, 1946), *Streptococcus oralis* (Bridges and Sneath, 1982), and *Streptococcus mitis* (Andrewes and Horder, 1906). *Int J Syst Bacteriol* **39**, 471–484.
- McKee, A. S., McDermid, A. S., Ellwood, D. C. & Marsh, P. D. (1985). The establishment of reproducible, complex communities of oral bacteria in the chemostat using defined inocula. *J Appl Bacteriol* **59**, 263–275.
- Pirt, S. J. (1975). Mixed cultures. In *Principles of Microbial and Cell Cultivation*, pp. 199–210. Oxford: Blackwell Scientific Publications.
- Shah, H. & Gharbia, S. E. (1992). Biochemical and chemical studies on strains designated *Prevotella intermedia* and proposal of a new pigmented species, *Prevotella nigrescens* sp. nov. *Int J Syst Bacteriol* **42**, 542–546.
- Sharon, N. & Lis, H. (1982). Glycoproteins. In *The Proteins*, vol. V, pp. 1–144. Edited by H. Neurath & R. L. Hill. London: Academic Press.
- Slots, J. (1981). Enzymatic characterization of some oral and nonoral gram-negative bacteria with the API ZYM system. *J Clin Microbiol* **14**, 288–294.
- Smith, K. & Beighton, D. (1986). The effect of the availability of diet on exoglycosidase production by supragingival plaque of macaque monkeys. *J Dent Res* **65**, 1349–1352.
- Smith, K. & Beighton, D. (1987). Proteolytic enzymes in the supragingival plaque of macaque monkeys. *Arch Oral Biol* **32**, 473–476.
- ter Steeg, P. F., van der Hoeven, J. S., de Jong, M. H., van Munster, P. J. J. & Jansen, M. J. H. (1988). Modelling the gingival pocket by enrichment of subgingival microflora in human serum in chemostats. *Microb Ecol Health Dis* **1**, 73–84.
- Veldkamp, H. (1976). Mixed culture studies with the chemostat. In *Continuous Culture 6: Applications and New Fields*, pp. 315–328. Edited by A. C. R. Dean, D. C. Ellwood, C. G. T. Evans & J. Melling. Chichester: Ellis Horwood.
- van der Hoeven, J. S. & Camp, P. J. M. (1991). Synergistic degradation of mucin by *Streptococcus oralis* and *Streptococcus sanguis* in mixed chemostat cultures. *J Dent Res* **70**, 1041–1044.

Received 5 May 1994; revised 8 July 1994; accepted 9 August 1994.