Antonie van Leeuwenhoek 53: 261–271 (1987)

Enrichment of subgingival microflora on human serum leading to accumulation of *Bacteroides* species, *Peptostreptococci* and *Fusobacteria*

P. F. TER STEEG[1], J. S. VAN DER HOEVEN[1], M. H. DE JONG[1], P. J. J. VAN MUNSTER[2] & M. J. H. JANSEN[2]

[1] *Department of Preventive and Community Dentistry, University of Nijmegen, P.O. Box 9101, NL-6500 HB Nijmegen, The Netherlands;* [2] *Clinical Chemical Laboratory, Department of Pediatrics, University Hospital, Nijmegen, The Netherlands*

Received 18 September; revised and accepted 16 April 1987

Key words: *Bacteroides, Peptostreptococcus, Fusobacterium*, periodontitis, protein degradation

Abstract. This study was undertaken to identify ecological factors that favour opportunistic pathogenic species in the subgingival microflora. In a first approach, human serum as a substitute for gingival exudate, was used for batch-wise enrichment of subgingival plaque. The microflora resulting after 5–6 enrichment steps consisted of black-pigmented and non-black-pigmented *Bacteroides* species, *Peptostreptococcus micros* and *Fusobacterium nucleatum* as the main organisms. It is noted that the same group of species was found to be enriched independent upon the origin of the subgingival plaque sample. It was suggested that these organisms are favoured by the increased flow of gingival exudate during inflammation.

The consortium of organisms was capable of selective degradation of serum (glyco-)proteins. Four different types of degradation occurred. After a prolonged period of growth complete degradation of immunoglobulins, haptoglobin, transferrin and complement C3c was observed. Partial degradation of immunoglobulins, haptoglobin, transferrin, albumin, alpha$_1$-antitrypsin and complement C3c and C4 was generally observed after 48 h of growth. Besides, immunoglobulin protease activity yielding Fc and Fab fragments was found. The consortium was also capable of consuming carbohydrate side-chains as indicated by an altered electrophoretic mobility of the serum glycoproteins.

Introduction

Periodontal disease is usually characterized by a complicated microflora consisting of a large variety of anaerobes (Moore et al. 1982). Very little is known about the ecological factors which lead to the establishment and maintenance of this pathogenic flora. Aim of this study was to find out which ecological factors favour opportunistic pathogenic organisms such as *Bacteroides* species (Loesche 1984; Slots 1982; Slots & Genco 1984; van Winkelhoff 1986) in the

oral cavity. Studies on the role of organisms and ecological factors have often employed enrichment strategies to identify the role of specific factors for the ecosystem (Beijerinck 1901; Harder & Dijkhuizen 1982). We suggested that gingival exudate is an ecological factor of importance in the subgingival ecosystem. In addition to a variety of host-defense factors, it presents a major source of substrates to the subgingival microflora, like saliva does to the supragingival plaque (De Jong et al. 1987). The chemical composition of the crevicular fluid shows an increasing resemblance to that of serum as soon as inflammation of the gingiva occurs (Bickel & Cimasoni 1986; Hattingh & Ho 1980). We accordingly used native human serum as a growth medium in batch-wise enrichments of the subgingival microflora under similar anaerobic conditions as exist in periodontal pockets.

Basically the strategy behind enrichment cultures is to select for bacteria that optimally utilize the available substrates (Beijerinck 1901; Brock et al. 1984). In batch-wise enrichment cultures this is achieved by selection in a series of sequential cultures on a liquid medium containing the specific substrate. The first step of the enrichment culture is inoculated with a sample from the microbial ecosystem to be investigated. In batch-wise enrichments organisms with a high growth-rate and adapted to growth at high substrate concentrations are favoured. Complex macromolecular substrates, such as serum, may well be utilized more completely by bacterial consortia than by pure cultures because of complementary substrate specificities and enzyme activities for substrate hydrolysis.

In this study, as a first approach, we have selected in batch-wise enrichment cultures for a group (consortium) of microorganisms originating from a complex periodontal microflora which are favoured by anaerobic conditions, a high growth-rate and an increased flow of gingival exudate (i.e. non-growth limiting amounts of serum as substrate).

Materials and methods

For this study we selected patients without symptoms of acute periodontitis. The pockets involved were untreated and 4–7 mm deep and the gingiva showed some bleeding upon probing. After removal of the supragingival plaque, subgingival plaque samples were taken with an extirpation needle and immediately transferred into an anaerobic chamber (Braun, Garching, West-Germany; 91% N_2, 5% CO_2, 4% H_2, 37°C). All further experimental procedures were carried out in this chamber. 0.5 ml of prereduced resuspension medium containing 10 $g.l^{-1}$ Tryptone (Difco), 10 $g.l^{-1}$ Yeast extract (Difco), 1.25 $g.l^{-1}$ $MgSO_4.7H_2O$, 1.25 $g.l^{-1}$ K_2HPO_4 and 2 $g.l^{-1}$ glucose, was added to the sample and it was vortexed for 30 s. Portions of 0.1 ml were inoculated into 5 ml batches of prereduced active human serum and repeatedly transferred (by 100-fold dilution into fresh

serum after 48 h of growth). The serum was provided by the local blood donor service and was obtained from 5 donors. Some potentially interesting parameters of the enrichment cultures were recorded at time of transfer. Optical densities (550 nm) were determined in a Zeiss PMQ3 spectrophotometer. The pH and redox-potential were measured with a combined pH and redox-electrode (Ingold). H_2S production was detected by blackening of lead-acetate paper.

Microbial analyses of the plaque samples and enrichment cultures included microscopical observations and cultural counts. Samples were serially diluted in resuspension medium and plated on prereduced blood-agar and BBM-medium (Gibbons & Macdonald 1960). Blood-agar contained 25 $g.l^{-1}$ Brain Heart Infusion (Difco), 10 $g.l^{-1}$ peptone (Difco), 20 $g.l^{-1}$ Bacto-Agar (Difco) and 1 $g.l^{-1}$ KNO_3, 100 $ml.l^{-1}$ defibrinated sheep blood was added after cooling to 56 °C. BBM-medium (Gibbons & Macdonald 1960) containing 50 $mg.l^{-1}$ of kanamycine was used to enumerate b.p. *Bacteroides*.

The dominant flora after 5–6 enrichment steps ($\pm$ 50 bacterial generations) was identified by randomly selecting 25 colonies from representative plates. In addition to this procedure differential counts based on colonial morphology were made using a stereo-microscope inserted in the window of the anaerobic chamber. Representative colonies were subcultured for further identification. From all isolates in both procedures Gram-stain and cellular morphology were determined. Gram-positive and negative rods were identified with the API-20A^R (anaerobes) (API system, S.A. Montalieu-Vercieu, France). Streptococci were identified using the API-20 Strep^R system. Weak Gram-positive peptostreptococci, Gram-negative cocci and some Gram-negative rods were identified with the API-20A^R system and the API-An-Ident system (API, Analytab Products, N.Y.). If necessary, additional tests were performed like growth on blood-agar aerobically, growth and fermentation products on PYG-broth (Holdeman et al. 1977). Several isolates were sent to the National Health Laboratories (RIVM) for identification and confirmation. Unknown *Bacteroides* species were sent to T. J. M. van Steenbergen (Oral Microbiology, Free University, Amsterdam) for identification. Degradation of serum proteins was investigated qualitatively with immunoelectrophoresis using respective antisera. Quantitative measurement of specific serum proteins was performed with semi-automated immunonephelometry using monospecific antisera raised in rabbits.

Results

In these experiments 3 samples of subgingival plaque were enriched on human serum. Samples A and B were removed from the same dental pocket with an interval of approximately 3 months time, whereas sample C originated from a different subject. All samples contained spirochetes (up to 10%) using dark-

field microscopy. Cultural counts showed low or non-detectable numbers of *b.p. Bacteroides.*

The composition of only one plaque sample, sample A was characterized in detail by picking random isolates. *Fusobacterium nucleatum* was the only species found among the random isolates from the original plaque sample and the resulting enrichment culture. A close relationship between the dominant microflora before and after enrichment of plaque samples, however, is not necessarily to be expected mainly because the conditions in the enrichment culture (i.e. growth at a maximal growth-rate for 50 bacterial generations) offer a strong selective pressure, favouring a small group of specialists among the periodontal microflora.

In the first steps of the batch-wise enrichment the optical densities at 550 nm (OD_{550}) of the cultures after 48 h of growth increased rapidly till a stable level at an OD_{550} of 15, was reached. The increase in OD_{550} coincided with a decline in redox-potential from –70 mV to –375 mV. Continued growth of the cultures till 72 h, instead of 48 h as used normally in these experiments, yielded cultures with an OD_{550} of 20–30 and redox-potentials of –400 mV to –550 mV. Strong H_2S production was always found. The initial pH of the serum was 7.8. In the first enrichment steps the pH dropped to approximately 6.4 after growth. Towards the end of a series of enrichment steps the pH initially decreased till 6.4 and then increased again to a value of 7.0.

After 5–6 steps of enrichment a population adapted to rapid growth on serum had developed. Comparison of the enriched flora from different pocket samples showed that microflora's with a similar composition were obtained (Table 1).

Peptostreptococcus micros became numerically dominant in the last 2–3 steps of the enrichments. The two non-black-pigmented *Bacteroides* species, *Bacteroides pneumosintes* and *Bacteroides* PS070, were difficult to distinguish on basis of colonial morphology and therefore not counted separately. *Bacteroides* species *PS070* was however detected most frequently in the random isolates and was besides *Ps. micros* the second dominant species in the enriched microflora. It fermented glucose, saccharose and maltose. One isolate was sent to T. J. M. van Steenbergen (Oral Microbiology, Amsterdam) who confirmed that it was a yet unnamed *Bacteroides* species. The identity of *Bacteroides pneumosintes* was confirmed by the RIVM (Bilthoven) and T. J. M. van Steenbergen. *Fusobacterium nucleatum* was found after every step of enrichment maximally making up 19% of the total CFU. *Lactobacillus acidophilus* and *Streptococcus sanguis* II (*mitior*) were consistently found though often in low proportions. *Actinomyces PS052* was only found in enrichments A and B. The identity of this organisms is still uncertain due to its poor growth on test-media, unless serum was added and its unusual enzymic profile. Gram-stain, colonial morphology, very weak sugar fermentation and the other characteristics point however to *Actinomyces israelii.* The category others was formed by incidentally encountered species such

as *Eubacterium lentum, Actinomyces odontolyticus, Pasteurella* species and *Propionibacterium acnes*. Spirochetes were found in the plaque samples but could not be detected in the enrichment cultures using interference contrast and dark-field microscopy.

Special attention was paid to the group of b.p. *Bacteroides* species. *Bacteroides intermedius* was consistently found after 2–3 steps of enrichment maximally making up 15% of the total CFU as can be seen in Table 2. In contrast, the levels of this organism in the original plaque samples were often below detection (<0.1%). Enrichment of *Bacteroides intermedius* coincided with redox-potentials (Eh) below −300 mV. *Bacteroides gingivalis* was not found in plaque samples and enrichment cultures. *Bacteroides melaninogenicus* was isolated only once.

The bacterial consortium in the enrichment culture degraded different serum (glyco-)proteins to a varying extent as is shown in table 3. Remarkably, albumin the main serum protein was degraded to a lesser degree than the serum glycoproteins. More extensive degradation was observed in a culture grown till the stationary phase (96 h, Fig. 1B) than in a culture grown for the normal 48 h (Fig. 1A). The immunoelectrophoretic patterns allowed to discern four different types of glycoprotein degradation as is shown in Fig. 1.

- Complete degradation of the immunoglobulins, haptoglobin, transferrin and complement C3c as is revealed by the absence of immune-precipitation after a prolonged period of incubation (Table 3, Figs. 1B1, 2).
- Extensive degradation of all investigated proteins resulting in decreases of the immunonephelometric reaction and/or distortion of the precipitation lines

Table 1. Composition of the dominant flora after enrichment of 3 subgingival plaque samples (A, B and C) on human serum (percentages of total CFU).

Species	A(5)*	B(6)	C(6)
Peptostreptococcus micros	42.9	43.8	58.0
Bacteroides sp.**	7.1	28.8	33.3
Bacteroides intermedius	7.1	4.2	2.2
Fusobacterium nucleatum	3.6	0.5	3.2
Lactobacillus acidophilus	17.9	5.5	1.0
Streptococcus sanguis II/I	3.6	2.8	1.0
Actinomyces PS0052	14.3	14.2	–
others	3.5	0.2	1.3
Number of isolates	28	24	34
CFU.ml^{-1}	$1.3.10^{10}$	$1.1.10^{10}$	$4.7.10^{9}$

* Number of enrichment steps in parentheses.

** Percentages of *Bacteroides pneumosintes* and *Bacteroides* sp. (PS070) were pooled because colonies could not be sufficiently discriminated.

Table 2. Detection of *Bacteroides intermedius* in subgingival plaques A, B and C and after enrichment of these samples on human serum (percentages of total CFU).

Sample		Percentage *B. intermedius*					
	Plaque	Enrichment step no.					
		1	2	3	4	5	6
A	N.D.*	N.D.	11.7	4.6	3.9	7.1	–
B	N.D.	N.D.	N.D.	9.8	14.7	–	5.2
C	0.6	N.D.	N.D.	0.2	7.4	13.7	2.2

* Detection level was approximately 0.1%. N.D. means no black-pigmented colonies detected on blood-agar and BBM-plates.

after immunoelectrophoresis (Table 3, Fig. 1).

- Immunoglobulin protease presumably cleaving into Fc and Fab fragments as described previously (Kilian 1981) (Fig. 1A2).
- Altered electrophoretic mobility due to removal of carbohydrate side-chains of glycoproteins (Fig. 1 A2–5).

As there is a shortage of blood donors and products in the Netherlands, it was checked whether other substrates could be substituted for human serum. Thus, in a pilot study periodontal plaque was enriched on calf serum, sheep serum, human plasma and dialyzed plasma (in order to remove part of the glucose and sodium citrate). The animal sera had to be discarded because b.p. *Bacteroides* were not or only incidentally found after enrichment. Enrichment on plasma resulted in a final pH of 5.0 to 5.5 and a microflora mainly consisting of *L. acidophilus* whereas in sera (see also Table 1) 6–8 species were found to be enriched.

We also tested the effect of using heat-inactivated (56 °C, 30 min) serum rather than active serum on the composition of the enrichment cultures in these pilot experiments. Heat-inactivation of the serum did not have any significant effect on the enriched microflora from periodontal pockets.

Fig. 1 A Immuno-electrophoretic patterns of serum-culture supernatants using polyvalent and monospecific antisera. The upper wells contained reference human serum, the lower wells culture supernatant – fifth step of an enrichment culture grown for 48 h, B – seventh step of an enrichment culture grown for 96 h. A1 – total proteins, A2 – extensive degration of immunoglobulins showing also cleavage in the hinge regions yielding Fc and Fab fragments with an altered electrophoretic motility due to removal of carbohydrate side chains, A3, B3 – extensive degradation of α_1-antitrypsin, A4, A5 – extensive degradation plus altered electrophoretic mobility of haptoglobin and transferrin respectively, B1 – total proteins, B2 – complete degradation of immunoglobulins, B3 – extensive degradation of α_1-antitrypsin.

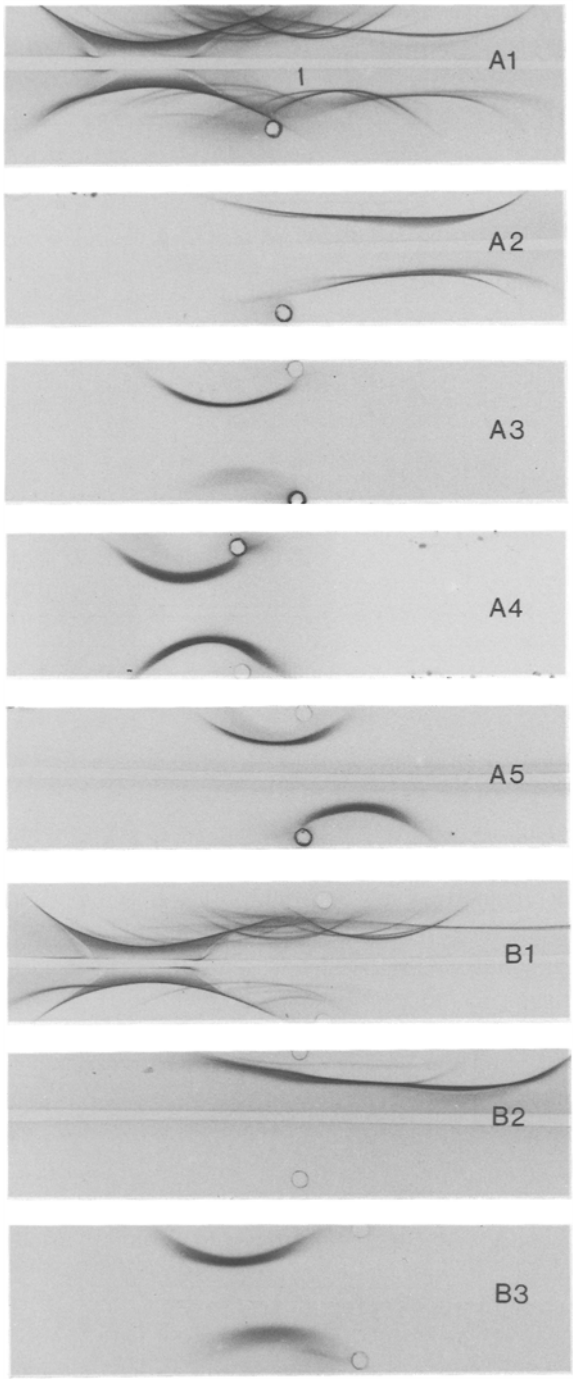
A1
1
A2
A3
A4
A5
B1
B2
B3

Table 3. Selective protein degradation after representative batch-wise enrichments of two subgingival plaque samples on human serum.

	Serum	% Degradation	
Proteins	$mg.l^{-1}$	step 5 (48 h)	step 7 (96 h)*)
albumin	43,000	20.9	17.7
transferrin	2,620	26.7	>90.5
haptoglobin	1,690	36.1	>99
complement C3c	920	53.4	>89.1
complement C4	290	41.4	20.7
α-1-antitrypsin	2,000	74.0	44.0
immunoglobulin G	10,000	69.6	100.0
immunoglobulin A	2,840	51.8	100.0
immunoglobulin M	1,590	71.7	100.0
total	64,950	33.9	42.8
gammaglobulins % normal values			
γ1 subclass	117	74.4	100.0
γ2 subclass	130	60.4	100.0
γ3 subclass	106	60.4	100.0
γ4 subclass	80	72.5	100.0

*) Step 7 of this batch-wise enrichment was prolonged to 96 h in contrary to the regular 48 h.

Discussion

As a first approach to identify ecological factors in the subgingival ecosystem we performed batch-wise enrichments of subgingival plaque on human serum. Batch-wise enrichments are likely to select for fast-growing primary and secondary utilizers of substrates in serum. In the batch culture substrates are in excess and these experimental conditions may, to a certain extent, reflect environmental conditions in the sulcus area of inflamed gingiva where the increased flow of gingival exudate in periodontal inflammation (Bickel & Cimasoni 1986; Hattingh & Ho 1980) presents a continuous supply of fresh substrates for the subgingival microflora. Our results (Table 1) show that these conditions lead to the accumulation of a typical and reproducible microflora. Dominant members of this microflora: *Peptostreptococcus micros*, *Bacteroides intermedius*, *Bacteroides pneumosintes* and *Fusobacterium nucleatum* are frequently found as predominant species in clinical infections and adult and juvenile periodontitis (Moore et al. 1981, 1982, 1983, 1985). These organisms may well benefit from inflammatory conditions resulting in increased flow of exudate.

The enrichment of *Bacteroides intermedius* is in accordance with the often found association of this organism with periodontal diseases (Loesche et al. 1985; Kornman & Loesche 1980; Slots 1982; Slots & Genco 1984). The observa-

tion that *B. intermedius* was only found in enrichment cultures with final redox-potentials below –300 mV deserves future attention. It is known that serum stimulates the growth of *B. intermedius* (Okuda et al. 1984). Little effect of serum on the growth of *Bacteroides gingivalis* was found (Okuda et al. 1984). *Bacteroides gingivalis* is absent in our enrichment cultures. Its presence in early onset periodontitis and its disappearance in later stages (Loesche et al. 1985) may suggest that it cannot benefit as well as *Bacteroides intermedius* of the increased flow of gingival exudate.

It is also of interest that *Peptostreptococcus micros*, the dominant microorganism in the enrichments, has recently been reported to possess several disease related characteristics including the production of hyaluronidase (Tam & Chan 1985; Tam et al. 1982). The presence of this organism in the subgingival microflora may well be underestimated in many studies due to the fact that colonies on blood-agar are small and inconspicuous and that mature cultures often stain Gram-negative.

The rapid disappearance of spirochetes in our enrichment cultures despite the fact that these organisms are ubiquitous in periodontal disease (Loesche & Laughon 1982; Mikx et al. 1986) may well be related to our experimental model which allows for selection of fast-growing organisms only. In contrast, spirochetes are rather slow growing micro-organisms (Mikx et al. 1982; Cheng & Chan 1983). Preliminary results of enrichments in continuous culture, allowing selection at low growth-rates (generation times of 7–9 h), have indicated the accumulation of *Treponema denticola*.

A further significance of our findings is, that we now have identified a set of oral bacteria whose concerted action enables them to grow on active serum and break down serum components almost completely. Preliminary findings showed that from the dominant members of the enrichment microflora only pure cultures of *P. micros* were capable of visible growth on active human serum as reflected by an OD_{550} after growth of 0.8 and 5.10^8 CFU.ml^{-1}. In our enrichment cultures *P. micros* reached 5.10^9 CFU.ml^{-1} showing that it also greatly benefited from the concerted action of the microflora in the enrichment culture.

The extensive or complete degradation of many serum proteins in our enrichment cultures is also indicative of the actions of consortia. Frandsen et al. (1986) reported that most oral bacteria are not or only partially capable of IgA degradation. Our observation that IgA and other host-defense factors such as complement C3c, IgG and IgM could be completely degraded in the enrichment cultures suggested that members of the consortium possess complementary enzymic activities. Glyco-proteins were prefentially consumed in the enrichment cultures as is apparent from the fact that albumin, a genuin protein and the main serum component, was hardly attacked. Haptoglobin and transferrin might be attractive substrates to the microflora both because of their carbohydrate and iron content, α_1-antitrypsin and γ-globulins also contain carbohydrates. Similarly de

Jong et al. (1987) observed a preference of oral bacteria for glycoproteins in enrichment cultures of supragingival plaque on saliva.

No effect of heat-inactivation of serum on the species composition after enrichment was found, which may indicate that consortia of microorganisms directly isolated from the gingival crevice were rather resistant to the bactericidal properties of gingival exudate or serum. Besides black-pigmented *Bacteroides* are known to possess several mechanisms to evade the bactericidal properties of serum (Nilsson et al. 1985; Sundqvist et al. 1985).

In summary, it may be concluded that besides its role in the host-defense response, gingival exudate plays also a major role as a substrate for the subgingival microflora. In relation with recent findings of de Jong et al. (1986) which showed that saliva is a major source of substrates for the supragingival microflora the results presented in this paper show the need for future studies on the role of host derived substrates in oral microbiology.

Acknowledgements

We thank Paul J. van Zutphen, Cees W. A. van den Kieboom and Peter J. M. Camp for technical assistance. Marianne van den Boogaard is acknowledged for typing the manuscript. T. J. M. van Steenbergen (Oral Microbiology, Amsterdam) and the RIVM (Bilthoven) are acknowledged for supporting bacterial identification.

References

Beijerinck, M. W. (1901) Anhaufungsversuche mit Ureumbakterien. Centralblatt f. Bakteriologie. II. 7: 33–61

Bickel, M. & G. Cimasoni (1986) Recent advances in gingival crevicular fluid (GCF) research. In: T. Lehner & G. Cimasoni (Eds) The Borderland between Caries and Periodontal Research III. (pp. 61–79) Academic Press, London

Brock, T. D., D. W. Smith & M. T. Madigan (1984) Biology of Microorganisms (pp. 641–642) Pretince-Hall Int., London

Cheng, S.-L. & E. C. S. Chan (1983) The routine isolation growth and maintenance of the intermediate-size anaerobic oral spirochetes from periodontal pockets. J. Periodont. Res. 18: 362–368

Frandsen, E. F. G., E. Theilade, B. Ellegaard & M. Kilian (1986) Proportions and identity of IgA1-degrading bacteria in periodontal pockets from patients with juvenile and rapidly progressive periodontitis. J. Periodont. Res. 21: 613–623

Gibbons, R. J. & J. B. Macdonald (1960) Hemin and vitamin K as required factors for cultivation of certain strains of *Bacteroides melaninogenicus*. J. Bacteriol. 80: 164–170

Harder, W. & L. Dijkhuizen (1982) Strategies of mixed substrate utilization in microorganisms. Phil. Trans. R. Soc. London. B. 297: 459–480

Hattingh, J. & E. Ho (1980) The concentration of proteins in human gingival crevicular fluid. J. Periodont. Res. 15: 90–95

Holdeman, L. V., E. P. Cato & W. E. C. Moore (Eds) Anaerobe Laboratory Manual (1977) 4th edn. Viriginia Polytechnic Institute and State University, Blacksburg

De Jong, M. H., J. S. van der Hoeven & J. H. van Os (1986) Growth of supragingival dental plaque on saliva-agar. J. Dent. Res. 65: 85–88

De Jong, M. H. & J. S. van der Hoeven (1987) The growth of oral bacteria on saliva. J. Dent. Res. 66: 498–505

Loesche, W. J. & B. E. Laughon (1982) Role of Spirochetes in Periodontal Disease. In: R. J. Genco & S. E. Mergenhagen (Eds) Host Parasite Interactions in Periodontal Diseases (pp. 62–75) ASM, Washington D.C.

Loesche, W. J. (1984) Possibilities for treating periodontal disease as specific anaerobic infections. J. Can. Dent. Assoc. 50: 467–472

Loesche, W. J., S. A. Syed, E. Schmidt & E. C. Morrison (1985) Bacterial profiles of subgingival plaques in periodontitis. J. Periodontol. 56: 447–456

Kilian, M. (1981) Degradation of immunoglobulin A1, A2 and G by suspected principal periodontal pathogens. Infect. Immun. 34: 757–765

Kornman, K. S. & W. J. Loesche (1980) the subgingival microbial flora during pregnancy. J. Periodont. Res. 15: 111–122

Mikx, F. H. M., T. Tonies, R. Verhoeven & T. Pijnenburg (1982) Biochemical and physiological characteristics of *Treponema denticola* isolated from ANUG lesions. J. Dent. Res. 62: 455 (58)

Mikx, F. H. M., M. I. Matee & J. C. Maltha (1986) The occurrence of oral spirochetes in relation to age and periodontal disease. In: T. Lehner & G. Cimasoni (ESD) The Borderland between Caries and Periodontal Research. III (pp. 169–177) Academic Press, London

Moore, W. E. C., E. P. Cato & L. V. Holdeman (1981) Anaerobes associated with periodontal disease. In: Specia Symposium Paris, 1980 (pp. 121–128). Les Anaerobies. Microbiologie-Pathologie, Masson, Paris

Moore, W. E. C., L. V. Holdeman, E. P. Cato, R. M. Smibert, J. A. Burmeister, K. G. Palcanis, & R. R. Ranney (1985) Comparative bacteriology of juvenile periodontitis. Infect. Immun. 48: 507–519

Moore, W. E. C., L. V. Holdeman, E. P. Cato, R. M. Smibert, J. A. Burmeister & R. R. Ranney (1983) Bacteriology of moderate (chronic) periodontitis in mature adult humans. Infect. Immun. 42: 510–515

Moore, W. E. C., L. V. Holdeman, R. M. Smibert, D. E. Hash, J. A. Burmeister & R. R. Ranney (1982) Bacteriology of severe periodontitis in young adult humans. Infect. Immun. 38: 1137–1148

Nilsson, T., J. Carlsson & G. Sundqvist (1985) Inactivation of key factors of the plasma proteinase cascade systems by *Bacteroides gingivalis*. Infect. Immun. 50: 467–471

Okuda, K., K. Ohta, T. Sasaki & I. Takazoe (1984) Morphological changes in the bacterium *Bacteroides melaninogenicus* subspecies *melaninogenicus* isolated from the human mouth and grown in culture without added blood components. Archs oral Biol. 29: 81–85

Slots, J. (1982) Importance of black-pigmented *Bacteroides* in human periodontal disease In: R. J. Genco & S. E. Mergenhagen (Eds) Host-Parasite Interactions in Periodontal Diseases (pp. 27–45) American Society for Microbiology, Washington, D.C.

Slots, J. & R. J. Genco (1984) Black-pigmented *Bacteroides* species, *Capnocytophaga* species, and *Actinobacillus actinomycetemcomitans* in human periodontal disease: Virulence factors in colonization, survival and tissue destruction. J. Dent. Res. 63: 412–421

Sundqvist, G., J. Carlsson, B. Hermann & A. Tärnvik (1985) Degradation of human immunoglobins G and M and complement factors C3 and C5 by black-pigmented *Bacteroides*. J. Med. Microbiol. 19: 85–94

Tam, Y.-C. & E. C. S. Chan (1985) Purification and characterization of hyaluronidase from oral *Peptostreptococcus* species. Infect. Immun. 47: 508–513

Tam, Y.-C., Harvey, R. F. & Chan, E. C. S. (1982) Chondroitin sulfatase-producing and hyaluroni-