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Biochemical and Serological Characterization of *Bacteroides intermedius* Strains Isolated from the Deep Periodontal Pocket

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Fifty-one fluorescence-positive black-pigmented *Bacteroides* strains obtained from 51 patients with deep periodontal pockets (>6 mm) were identified and characterized. Fifty of these strains were presumptively identified as *Bacteroides intermedius* according to the indole reaction. This was confirmed by further biochemical characterization. The 50 strains from diseased sites were then compared with 16 *B. intermedius* strains isolated from periodontally healthy individuals with no signs of destructive periodontal disease. Tests for antimicrobial susceptibility showed similar patterns for all 50 pocket-derived strains, except for one β-lactamase-positive strain that was resistant to penicillin G and ampicillin. Forty-seven strains were tested for binding of three monoclonal antibodies defining three distinct serogroups of *B. intermedius*. Thirty-one strains belonged to serogroup I, three to serogroup II and thirteen to serogroup III. In comparison to the strains from the shallow periodontal pockets, serogroup I was significantly overrepresented in the patient group with periodontal disease. We conclude that saccharolytic black-pigmented *Bacteroides* species from deep periodontal pockets constituted, with very rare exceptions, a biochemically homogeneous but antigenically heterogeneous group of *B. intermedius* and that serogroup I is predominantly found in deep periodontal lesions.

The group of black-pigmented *Bacteroides* species has received much attention in studies of rapidly progressing periodontitis in adults (16, 19, 28, 34). *Bacteroides gingivalis*, a black-pigmented *Bacteroides* species now termed *Porphyromonas gingivalis*, which does not show fluorescence in long-wave UV light, has in particular been associated with active disease and loss of periodontal attachment (23, 28), while fluorescence-positive species have been more difficult to associate with the disease. Species of the *Bacteroides melaninogenicus* group may occasionally occur in deep pockets of advanced cases (31). However, this group has rather been associated with locations such as dorsum of tongue, tonsils, and oral mucosa in both periodontally diseased and healthy individuals as well as with the gingival margins of individuals with inadequate oral hygiene (30). *Bacteroides intermedius* has been found in both periodontally diseased and healthy patients on oral mucosa, the tongue, and tonsils, as well as in the subgingival plaque of deep periodontal pockets (30, 31). Some studies also report a high frequency and positive association of *B. intermedius* strains with periodontal breakdown (26, 32, 34). It has been suggested (7, 18) that the species with the epithet *B. intermedius* includes several biochemical, serological, and genotypical variants. This may explain in part the somewhat contradictory appearance of this species in both healthy and diseased sites. The present study deals with the biochemical and serological characterization of fluorescence-positive black-pigmented *Bacteroides* strains from deep periodontal pockets in comparison with strains isolated from individuals with no signs of destructive periodontal disease.

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MATERIALS AND METHODS

Bacterial strains. Fifty-one strains of black-pigmented *Bacteroides* species, all from different individuals, were collected from samples taken from patients with moderate to advanced periodontal tissue breakdown (22a). All sites sampled showed a probing pocket depth of more than 6 mm and exhibited clinical signs of inflammation such as bleeding on probing or suppuration. The samples contained a predominant number (>0.5%) of black-pigmented *Bacteroides* sp., which was not identified as *P. gingivalis* in a preliminary identification test. In addition, 16 *B. intermedius* strains were identified from subgingival samples from shallow pockets (≤3 mm) in subjects without signs of destructive periodontal disease. Type strain *B. intermedius* NCTC 9336 was used as reference strain.

Isolation and preliminary identification. Three paper points, inserted to the bottom of a selected periodontal pocket for 15 s, were used for sampling. The paper points were transferred to a vial containing 3.3 ml of transport medium VMGA III (17) and were processed within 24 h. The samples were thoroughly mixed and diluted to 10^{-2} and 10^{-4} by using VMG I (17). Samples (0.1 ml) of each dilution were uniformly spread onto brucella (BBL Microbiology Systems, Cockeysville, Md.) agar plates enriched with 5% defibrinated horse blood, 0.5% hemolyzed horse blood and 5 mg of menadione per liter and onto Trypticase soy agar with 75 mg of bacitracin per liter and 5 mg of vancomycin per liter (25).

The total viable count was recorded after 7 to 9 days of anaerobic incubation in jars by using the hydrogen combus-

TABLE 1. Bacterial recovery in 51 samples from periodontally diseased individuals and 16 samples from individuals without signs of destructive periodontal disease[a]

Bacterium	Bacterial recovery					
	Diseased subjects			Nondiseased subjects		
	No. of positive samples (%)	Mean (%)	Range (%)	No. of positive samples (%)	Mean (%)	Range (%)
B. intermedius	50 (98)	13	0.8–34	16 (100)	5.3	0.2–15.9
B. melaninogenicus	1 (2)	<0.1		0	0	0
P. gingivalis	19 (37)	23	1.7–63	0	0	0
A. actinomycetemcomitans	18 (35)	1.1	0.1–5.2	6 (35)	10	0.0001–45
Total viable counts		$11 \cdot 10^6$	$0.15 \cdot 10^6$–$77 \cdot 10^6$		$6 \cdot 10^6$	$0.19 \cdot 10^6$–$20 \cdot 10^6$

[a] All samples contained *B. intermedius*-like bacteria.

tion method. Representative pigmented colonies were stained by Gram's method and suspended in a few drops of methanol to check red fluorescence under long-wave (360-nm) UV light (27) and to perform selected biochemical tests including glucose and lactose fermentation and indole production (14).

Porphyromonas gingivalis was identified as gram-negative rods growing as light or dark green or greenish black colonies on brucella blood agar. The cells did not exhibit fluorescence and failed to ferment glucose or lactose.

Bacteroides intermedius was identified as gram-negative rods growing as black colonies and exhibiting red fluorescence, they were indole positive and capable of fermenting glucose but not lactose.

Other pigmented colonies of gram-negative rods that did not have the above characteristics were primarily among the *B. melaninogenicus* group.

Actinobacillus actinomycetemcomitans was identified on the Trypticase soy agar plate described above as adherent small colonies giving a positive catalase reaction.

Gas chromatography. Analyses of metabolic products detected in peptone yeast medium with 1% glucose (PYG) were done by using gas-liquid chromatography (Sigma 2B [Perkin-Elmer, Norwalk, Conn.] equipped with a flame ionization detector) as outlined in the Virginia Polytechnic Institute manual (12). The glass column for the chromatography was packed with 5% AT 1000 (Altech Associates Inc., Deerfield, Ill.) on Chromosorb GHP 100/120 mesh (Johns-Manville, Denver, Colo.). The carrier gas was nitrogen (30 ml/min), the injection port temperature was 150°C, and the oven temperature was 120°C. One-microliter portions of the ether-extracted and methylated samples (12) were used, and the results were compared with those obtained with standard solutions of volatile fatty acids.

Biochemical tests. PY medium broth (12) was used as basal medium for fermentation of carbohydrates and derivatives. The preparation and inoculation of fermentation tubes were done in a series of biochemical reactions tested according to the Virginia Polytechnic Institute manual (12). The results were recorded as no reaction, weak reaction, or strong reaction (see Table 2).

API-ZYM tests. The API-ZYM colorimetric kit system (API System, La Balmes les Grottes, Montalieu-Vercieu, France) for detection of enzymes was used according to the directions of the manufacturer. Color reactions were read with a graded scale in which 0 indicated no enzyme activity, 1 and 2 indicated weak activity and 3 through 5 indicated strong enzyme activity.

Antimicrobial susceptibility test. Both agar plate dilution and the disk method as described by Holdeman et al. (12) were used for antimicrobial susceptibility testing. The agents tested included penicillin G (Astra, Södertälje, Sweden), ampicillin (Astra), tetracycline (Kabi Vitrum AB, Stockholm, Sweden), doxycycline (Pfizer AB, Täby, Sweden), minocycline (Sigma Chemical Co., St. Louis, Mo.), clindamycin (Sigma), kanamycin (Sigma), vancomycin (Sigma), erythromycin (Sigma), polymyxin B (Sigma), metronidazole (Sigma), and tinidazole (Pfizer). Nitrocefin (Glaxo Ltd., Greenford, Middlesex, England) was used for test of β-lactamase production.

Serotyping by using monoclonal antibodies. The strains were cultured by using brain heart infusion broth (BBL) enriched with 2 mg of hemin per liter and 1 mg of menadione per liter in an anaerobic chamber with an atmosphere of 85% N_2–10% CO_2–5% H_2 at 37°C for 5 days. After centrifugation at 3,000 rpm (Labor Wifug, AB Winkelcentrifug, Stockholm, Sweden) for 20 min, the bacterial cells were stored at −20°C until used. Immediately after defrosting, aliquots of the bacterial suspensions were transferred to Eppendorf microcentrifuge tubes, diluted with Dulbecco phosphate-buffered saline, sonicated for 2 to 3 s with a Branson B-12 Sonifier, and then centrifuged for 2 min in an Eppendorf 5415 centrifuge. The cells were washed three times with phosphate-buffered saline, sonicated again for 2 to 3 s, and suspended in 0.9% NaCl. The bacterial suspensions were microscopically controlled for proper dilution and then used to coat 15-well multitest slides (Flow Laboratories, Irvine, Scotland) with 10 μl per well. The air-dried and methanol-fixed bacteria were serogrouped by indirect immunofluorescence (33) by using the rat monoclonal antibodies 37BI6.1, 38BI1, 39BI1.1, and 40BI3.2 (7, 9) as first reagents and biotin-conjugated goat anti-rat immunoglobulin G (H+L) antibodies (Sigma) as second reagents. As a negative control, the *Actinomyces viscosus*-specific rat monoclonal antibody 31AV1.2 was included in each test.

All strains were tested at least twice. Each experiment was scored independently by two investigators. Every slide contained 10 test strains, 5 reference strains representing three *B. intermedius* serogroups (ATCC 25611^T from serogroup I, ATCC 25261 from serogroup II, and H187 from serogroup III), *B. melaninogenicus* (VPI 9343), and *A. viscosus* (Ny 1).

RESULTS

According to the selection criteria, all 51 sites with a deep periodontal pocket contained black-pigmented *Bacteroides* species, not including *P. gingivalis*, in a range of 0.8 to 34.3% of the total viable count. The mean total viable count was 11 × 10^6 CFU/ml of transport medium (Table 1).

Actinobacillus actinomycetemcomitans was found in 18 sites, accounting on the average for 1.1% of the total CFU.

Bacteroides strains were found in 19 sites and constituted 1.7 to 63.0% of the flora. Seven samples contained both *A. actinomycetemcomitans* and *P. gingivalis*. Of the 51 isolated strains, 50 were presumptively identified as strains of *B. intermedius* and one as *B. melaninogenicus*. For further characterization, we focused on the 50 *B. intermedius* strains.

In the 16 samples from subjects without signs of destructive periodontal disease and in which *B. intermedius* had been identified, *B. intermedius* constituted, on the average, 5.3% of the flora. The mean viable count was 6×10^6 CFU/ml of transport medium (Table 1). *A. actinomycetemcomitans* was found in six samples, while *P. gingivalis* was not found in these samples.

Colony appearance. After 7 days of growth on brucella agar, the colony appearance of most black-pigmented strains from deep periodontal pockets was smooth and regular. One strain showed an irregular edge, one strain was adherent, and one strain had both of these characteristics. Colonies of one strain were only weakly pigmented. Twenty-six of fifty strains had colonies that were hard and brittle, while the other strains had colonies that were mucoid and elastic. The strains from shallow pockets of the nondiseased individuals were colonially similar to strains isolated from deep periodontal pockets.

Gas chromatography. All strains produced acetic, isovaleric, and succinic acids in the ratio 3:1:1 when cultured in PYG. Most strains also produced isobutyric and lactic acid in minute amounts. No phenylacetic acid was detected.

Biochemical characteristics. The biochemical reactions of the 50 *B. intermedius* strains from deep periodontal pockets are shown in Table 2. They all produced indole and fermented glucose, glycogen, and maltose. Acid was produced from dextran, starch, and sucrose, while fermentation of fructose, mannose, rhamnose, and ribose was variable. Two strains fermented lactose. Esculin was not hydrolyzed. Most strains hydrolyzed cysteine, ornithine, and urea. Twenty-three strains did not digest gelatin. Milk was curdled by 23 strains and digested by 10 strains. The 16 strains from subjects with shallow pockets were all indole positive and fermented glucose but not lactose.

Enzyme pattern. Forty-seven strains from deep periodontal pockets were tested (Table 3). All strains showed strong alkaline and acid phosphatase and α-glucosidase. All strains were positive for phosphoaminidase. Weak esterase and esterase-lipase activity was demonstrated by 30 and 43 strains, respectively. Eighteen strains were chymotrypsin positive, and seventeen strains were fucosidase positive. Nine strains showed a strong β-galactosidase reaction. All strains were negative for *N*-acetyl-β-glucosaminidase. The 16 strains from subjects with shallow pockets showed an enzyme activity pattern similar to those of strains from subjects with deep periodontal pockets. One of these strains was positive for *N*-acetyl-β-glucosaminidase. No correlation was found between serogroup classification and enzyme activity pattern.

Antimicrobial susceptibility. Table 4 shows the antimicrobial susceptibility of 50 strains of *B. intermedius* as assessed by the agar dilution method. One strain showed resistance (>25 mg/ml) to penicillin G and ampicillin (Table 4), which was confirmed by the antibiotic disk method. A positive nitrocefin reaction indicated β-lactamase production by this strain.

Serology. All 47 tested strains from deep periodontal pockets exhibited cell wall fluorescence when labeled with monoclonal antibody 38BI1 (Table 5), which has been reported to bind to all saccharolytic black-pigmented *Bacteroides* strains (7, 9).

Of the 47 *B. intermedius* strains, 31 belonged to serogroup I (characterized by the binding of monoclonal antibodies 37BI6.1, 39BI1.1, and 40BI3.2). Only three strains fell into serogroup II (37BI6.1 and 39BI1.1 positive and 40BI3.2

TABLE 2. Biochemical reactions of 50 *B. intermedius* strains

Characteristic	No. of strains with		
	Strong reaction	Weak reaction	No reaction
Acid produced from:			
Adonitol	0	0	50
Amygdalin	0	0	50
Arabinose	0	0	50
Cellobiose	0	2	48
Dextran	12	36	2
Dulcitol	0	2	48
Esculin	0	0	50
Fructose	5	19	26
Galactose	0	5	45
Glucose	47	3	0
Glycerol	1	7	42
Glycogen	13	37	0
Inositol	0	4	46
Inulin	9	36	5
Lactose	2	0	48
Levan	4	0	46
Maltose	15	35	0
Mannitol	0	4	46
Mannose	0	19	31
Melezitose	0	1	49
Melibiose	0	5	45
Raffinose	1	5	44
Rhamnose	8	35	7
Ribose	2	11	37
Salicin	0	1	48
Sorbitol	0	0	50
Starch	10	40	0
Sucrose	23	27	0
Trehalose	1	7	42
Xylose	0	1	49
Bile tolerance	0	0	50
Growth stimulation of:			
Formate	0	0	50
Fumarate	0	0	50
Formate+fumarate	4	0	46
Hydrolysis of:			
Lysine	2	0	48
Cysteine	43	2	5
Ornithine	27	4	19
Urea	1	35	14
Arginine	10	6	34
Hippurate	0	0	50
Esculin	0	0	50
Production of:			
Indole	50	0	0
Hydrogen	0	0	50
Acetyl methyl carbinol	4	1	45
H_2S	18	5	27
Reduction of nitrate	15	1	34
Digestion of gelatin	26	1	23
Milk reaction			
Acid production	0	0	50
Curd	10	13	27
Digestion	6	4	40
Reduction	9	1	40

TABLE 3. Enzymatic characterization by the API-ZYM system of *B. intermedius* strains isolated from 47 periodontally diseased and 16 nondiseased sites

Enzyme	No. of strains from diseased sites with:			No. of strains from nondiseased sites with:		
	Strong reaction	Weak reaction	No reaction	Strong reaction	Weak reaction	No reaction
Alkaline phosphatase	47	0	0	16	0	0
Butyrate esterase	0	30	17	0	16	0
Caprylate esterase lipase	0	42	4	0	15	1
Myristase lipase	0	0	47	0	0	16
Leucine aminopeptidase	0	0	47	0	0	16
Valine aminopeptidase	0	0	47	0	0	16
Cystein aminopeptidase	0	0	47	0	0	16
Trypsin	0	0	47	0	0	16
Chymotrypsin	2	16	29	0	1	15
Acid phosphatase	47	0	0	16	0	0
Phosphoamidase	46	1	0	12	4	0
α-Galactosidase	0	0	47	0	1	15
β-Galactosidase	9	9	38	2	0	14
β-Glucuronidase	0	0	47	0	0	16
β-Glucosidase	47	0	0	12	4	0
α-Glucosidase	0	0	47	0	0	16
N-Acetyl-β-glucosaminidase	0	0	47	1	0	15
α-Mannosidase	0	0	47	0	0	16
α-Fucosidase	5	12	30	0	9	7

negative), while 13 were assigned to serogroup III (only 37BI6.1 positive). One strain did not fit into the scheme of these three serogroups. This strain was considered a variant of a serogroup I strain lacking the antigen detected by 39BI1.1. Of the 16 strains from individuals with shallow periodontal pockets, 5 were found to belong to serogroup I, 4 to serogroup II, and 7 to serotype III. The frequency of serogroup I strains isolated from deep pockets was higher and differed significantly ($P < 0.01$; chi-square test) from that of serogroup I strains isolated from healthy shallow sites.

There was no apparent relationship between the biochemical characteristics and the serological classification of the strains.

DISCUSSION

Among the black-pigmented *Bacteroides* species colonizing the human periodontium, the *B. melaninogenicus* group, including the species *B. melaninogenicus*, *B. denticola*, and *B. loescheii*, is frequently isolated from the oral cavities of periodontally healthy patients but seldom from deep periodontal pockets (30, 31, 34). Thus, the *B. melaninogenicus* group seems not to be associated with periodontal disease. The present study, in which only one strain of the *B. melaninogenicus* group was isolated from 51 deep periodontal pockets harboring saccharolytic black-pigmented *Bacteroides* strains, confirms that the deep periodontal pocket in humans is not the habitat of the three above-mentioned species. On the other hand, the nonfermenting, strongly proteolytic species *P. gingivalis* seldom has been found in plaque from the gingival crevices of periodontally healthy individuals (23, 34) but has been isolated from deep periodontal pockets, thus suggesting that the deep lesion is the major residence of this species in the human oral cavity.

B. intermedius is present in various oral locations both in children and in adults and colonizes healthy as well as diseased periodontal sites (5, 30, 31, 34). Specifically, this species has been associated with gingivitis in pregnant women (13) and acute necrotizing ulcerating gingivitis, which might indicate a pathogenic role (15). The widespread distribution of this species could be explained by its broad biochemical activity. The ability to ferment carbohydrates may favor its growth on teeth or oral mucosal surfaces, while the production of proteolytic enzymes may promote its growth in an environment where only small amounts of carbohydrates are available, such as gingival pockets. In the present study, *B. intermedius* frequently was isolated from

TABLE 4. MICs of selected antimicrobial agents for 50 isolates of *B. intermedius* tested by the agar dilution method

Antimicrobial agent	No. of susceptible strains at the following MIC (μg/ml):									
	≥100	50	25	10	5	1	0.5	0.1	0.05	≤0.01
Penicillin	0	0	1	0	0	0	0	0	34	15
Ampicillin	0	0	1	0	0	0	0	5	44	0
Tetracycline	0	0	0	0	1	0	40	9	0	0
Doxycycline	0	0	0	0	0	0	0	7	37	6
Minocycline	0	0	0	0	0	0	1	0	49	0
Clindamycin	0	0	0	0	0	0	0	0	0	50
Kanamycin	48	1	1	0	0	0	0	0	0	0
Vancomycin	14	20	14	1	1	0	0	0	0	0
Erythromycin	0	0	0	0	0	0	7	30	13	0
Polymyxin B	0	0	0	0	4	20	26	0	0	0
Metronidazole	0	0	0	0	11	2	36	0	1	0
Tinidazole	0	0	0	0	0	0	39	10	0	1

TABLE 5. Serogrouping of *B. intermedius* strains

B. intermedius serogroup[a]	No. of strains (%) from subgingival plaque of subjects	
	With periodontal disease	Without periodontal disease
I	31[b] (66)	5 (25)[c]
II	3 (6)	4 (31)
III	13 (28)	7 (44)

[a] The definition of the serogroups is given in the text.

[b] One strain reacted with monoclonal antibodies 37BI6.1 and 40BI3.2 but failed to react with 39BI1.1.

[c] Statistically significantly ($P < 0.01$, by the x^2 test) different from the proportion observed in diseased sites.

both deep and shallow sites. However, the enzyme profiles of the strains from different origins were nearly identical, indicating that the enzyme activity of these strains could not explain the colonization pattern of *B. intermedius*.

Analyses of the humoral host response against *B. intermedius* have shown similar antibody levels in individuals with and without periodontal disease (4, 8, 28, 29). This lack of association with periodontal disease could be explained in part by the frequent colonization of *B. intermedius* in locations other than the gingival pocket. However, they may also be related to the extensive antigenic heterogenicity among *B. intermedius* strains (6), which so far has only been partially elucidated (7, 21). The serogroup analysis of this investigation support the results of Gmür and Guggenheim (7) and Gmür and Wyss (9), who found that all *B. intermedius* isolates can be assigned to one of three serogroups. Again, strains of all serogroups colonized the deep periodontal pocket. Additionally, serogroup I strains were encountered significantly more often in deep pockets than in sites from individuals without signs of destructive periodontal disease. As serogroup I strains most likely belong to the genotype I of the species, while serogroup II and III strains are of genotype II (9), these results corroborate the observation of Moore et al. (20) of a preferential association of genotype I strains with deep periodontal lesions.

This study further supports phenotypical homology among the strains from different serogroups, which means that variations in the biochemical or enzymatic characteristics of the strains could not be related to the serogroup classification. This does not, however, exclude the possibility that various characteristics may exist that also may influence the pathogenicity of *B. intermedius* strains.

Carbohydrate fermentation tests and API-ZYM analysis resulted in an overall confirmation of previous reports (7, 10, 11, 12, 24, 31). Two strains were lactose positive, which is notable since this reaction is proposed to discriminate *B. intermedius* isolates from other black-pigmented *Bacteroides* strains with a positive fluorescence reaction (27). Lactose cleavage by β-galactosidase has been used, for example, to rapidly distinguish *B. intermedius* from *B. melaninogenicus*, *B. denticola*, or *B. loescheii* colonies on MUG (4-methylumbelliferyl-β-D-galactoside) agar plates (1). Since both lactose fermentation and β-galactosidase production have been observed to be uncertain criteria in other studies (7, 23), we suggest that other enzymes such as *N*-acetyl-β-glucosaminidase should preferentially be incorporated when characterizing these species.

The patterns of susceptibility to antimicrobial agents were extremely homogeneous. Only one strain was found to be β-lactamase positive and resistant to penicillin and ampicillin as measured with both the disk method and the agar dilution method. All other strains were susceptible to less than 0.1 μg/ml, which is lower than what Brown and Waatti (3) and Baker et al. (2) have reported. This may reflect a true difference between the studies performed in the United States and the present investigation on strains isolated in Sweden. A German study (22) had shown an intermediate pattern. All strains except one were found to be susceptible to 0.5 μg or less of tetracycline and its derivatives per ml. This dose is lower than the one determined by Baker et al. (2), but it is in concordance with that of Niederau et al. (22) and Brown and Waatti (3).

In conclusion, it seems that the deep periodontal pocket harbors *B. intermedius* as the only saccharolytic black-pigmented *Bacteroides* strain. Serogroup I seems to be overrepresented in these isolates compared with isolates from shallow pockets. The isolates are otherwise phenotypically indistinguishable and show a homogeneous antibiotic susceptibility pattern.

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LITERATURE CITED

1. **Alcoforado, G. A. P., T. L. McKay, and J. Slots.** 1987. Rapid method for detection of lactose fermenting oral microorganisms. Oral Microbiol. Immunol. **2**:25–38.
2. **Baker, P. J. R., T. Evans, J. Slots, and R. J. Genco.** 1985. Susceptibility of human oral anaerobic bacteria to antibiotics suitable for topical use. J. Clin. Periodontol. **12**:201–208.
3. **Brown, W. J., and P. E. Waatti.** 1980. Susceptibility testing of clinically isolated anaerobic bacteria by an agar dilution technique. Antimicrob. Agents Chemother. **17**:629–635.
4. **Doty, S. L., D. E. Lopatin, S. A. Syed, and F. N. Smith.** 1982. Humoral immune response to oral microorganisms in periodontitis. Infect. Immun. **37**:499–505.
5. **Frisken, K. W., J. R. Tagg, A. J. Laws, and M. B. Orr.** 1987. Suspected periodontopathic microorganisms and their oral habitats in young children. Oral Microbiol. Immunol. **2**:60–64.
6. **Gmür, R.** 1985. Human serum antibodies against *Bacteroides intermedius*. Antigenic heterogeneity impairs the interpretation of the host response. J. Periodontal Res. **20**:492–496.
7. **Gmür, R., and B. Guggenheim.** 1983. Antigenic heterogenicity of *Bacteroides intermedius* as recognized by monoclonal antibodies. Infect. Immun. **42**:459–470.
8. **Gmür, R., K. Hrodek, U. P. Saxer, and B. Guggenheim.** 1986. Double-blind analysis of the relation between adult periodontitis and systemic host response to suspected periodontal pathogens. Infect. Immun. **52**:768–776.
9. **Gmür, R., and C. Wyss.** 1985. Monoclonal antibodies to characterize the antigenic heterogenicity of *Bacteroides intermedius*, p. 91–119. *In* A. L. J. Macario and E. Conway de Macario (ed.), Monoclonal antibodies against bacteria, vol. I. Academic Press, Inc., New York.
10. **Harding, G. K. M., V. L. Sutter, S. M. Finegold, and K. S. Bricknell.** 1976. Characterization of *Bacteroides melaninogenicus*. J. Clin. Microbiol. **4**:354–359.
11. **Hofstad, T.** 1980. Evaluation of the API ZYM system for identification of *Bacteroides* and *Fusobacterium* species. Med. Microbiol. Immunol. **168**:173–177.
12. **Holdeman, L., E. P. Cato, and W. E. C. Moore.** 1975. Anaerobe laboratory manual. Anaerobe laboratory, Virginia Polytechnic Institute and State University, Blacksburg.
13. **Kornman, K. S., and W. J. Loesche.** 1980. The subgingival microflora during pregnancy. J. Periodontal Res. **15**:111–122.
14. **Krieg, N. R., and J. G. Holt (ed.).** 1984. Bergey's manual of systemic bacteriology, vol. 1. The William & Wilkins Co., Baltimore.
15. **Loesche, W. J., S. A. Syed, B. G. Laughon, and J. Stoll.** 1982. The bacteriology of acute necrotizing ulcerative gingivitis. J. Periodontol. **53**:223–230.
16. **Loesche, W. J., S. A. Syed, E. Schmidt, and E. C. Morrison.** 1985. Bacterial profiles of subgingival plaque in periodontitis. J. Periodont. **56**:447–456.
17. **Möller, Å. J. R.** 1966. Microbiological examination of root canals and periapical tissue of human teeth. Thesis. Odontol. Tidskr. **74**:1–380.
18. **Moore, W. E. C.** 1987. Microbiology of periodontal disease. J. Periodontal Res. **22**:335–341.
19. **Moore, W. E. C., L. V. Holdeman, E. P. Cato, I. J. Good, E. P. Smith, R. R. Ranney, and K. G. Palcanis.** 1984. Variation in periodontal flora. Infect. Immun. **46**:720–726.
20. **Moore, W. E. C., L. V. Holdeman, R. M. Smibert, D. E. Hash, J. A. Burmeister, and R. R. Ranney.** 1982. Bacteriology of severe periodontitis in young adult humans. Infect. Immun. **38**:1137–1148.

21. **Nakazawa, F., J. J. Zambon, H. S. Reynolds, and R. J. Genco.** 1988. Serological studies on oral *Bacteroides intermedius*. Infect. Immun. **56**:1647–1651.
22. **Niederau, W., V. Höffler, and G. Pulverer.** 1980. Susceptibility of *Bacteroides melaninogenicus* to 45 antibiotics. Chemotherapy **26**:121–127.
22a. **Renvert, S., M. Wikström, G. Dahlén, J. Slots, and J. Egelberg.** 1990. Effect of root debridement on the elimination of *Actinobacillus actinomycetemcomitans* and *Bacteroides gingivalis* from periodontal pockets. J. Clin. Periodontal. **17**:345–350.
23. **Slots, J.** 1979. Subgingival microflora and periodontal disease. J. Clin. Periodontol. **6**:351–382.
24. **Slots, J.** 1981. Enzymatic characterization of some oral and nonoral gram-negative bacteria with the API ZYM system. J. Clin. Microbiol. **14**:288–294.
25. **Slots, J.** 1982. Selective medium for isolation of *Actinobacillus actinomycetemcomitans*. J. Clin. Microbiol. **15**:606–609.
26. **Slots, J., L. Bragd, M. Wikström, and G. Dahlén.** 1986. The occurrence of *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis* and *Bacteroides intermedius* in adults. J. Clin. Periodontol. **13**:570–577.
27. **Slots, J., and H. S. Reynolds.** 1982. Long-wave UV-light fluorescence for identification of black-pigmented *Bacteroides* spp. J. Clin. Microbiol. **16**:1148–1151.
28. **Tanner, A. C. R., S. S. Socransky, and J. M. Goodson.** 1984. Microbiota of periodontal pockets losing crestal alveolar bone. J. Periodontal Res. **19**:279–291.
29. **Taubman, M. A., J. L. Ebersole, and D. J. Smith.** 1982. Association between systemic and local antibody and periodontal diseases, p. 283–298. *In* R. J. Genco and S. E. Mergenhagen (ed.), Host-parasite interactions in periodontal diseases. American Society for Microbiology, Washington, D.C.
30. **Van der Velden, V., A. J. van Winkelhoff, F. Abbas, and J. de Graaff.** 1986. The habitat of periodontopathic microorganisms. J. Clin. Periodontol. **13**:243–248.
31. **Van Winkelhoff, A. J., V. Van der Velden, E. G. Winkel, and J. de Graaff.** 1986. Black-pigmented Bacteroides and motile organisms on oral mucosal surfaces in individuals with and without periodontal breakdown. J. Periodontal Res. **21**:434–439.
32. **Wennström, J., G. Dahlén, J. Svensson, and S. Nyman.** 1987. *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis* and *Bacteroides intermedius*. Predictors for periodontal disease? Oral Microbiol. Immunol. **2**:158–163.
33. **Werner-Felmayer, G., B. Guggenheim, and R. Gmür.** 1988. Production and characterization of monoclonal antibodies against *Bacteroides forsythus* and *Wolinella recta*. J. Dent. Res. **67**:548–553.
34. **Zambon, J. J., H. S. Reynolds, and J. Slots.** 1981. Black-pigmented Bacteroides spp. in the human oral cavity. Infect. Immun. **32**:198–203.