JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1979, p. 550-556
0095-1137/79/10-0550/07$02.00/0

Vol. 10, No. 4

# Synonymy of Strains of Center for Disease Control Group DF-1 with Species of *Capnocytophaga*

B. L. WILLIAMS,[1]* D. HOLLIS,[2] AND L. V. HOLDEMAN[3]

*Department of Periodontics, School of Dentistry, University of Washington, Seattle, Washington 98195[1]; Center for Disease Control, Atlanta, Georgia 30333[2]; and Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061[3]*

Received for publication 29 June 1979

Of eight strains of Center for Disease Control group DF-1 examined, seven had 62 to 87% deoxyribonucleic acid homology with the neotype strain of *Capnocytophaga ochracea* and one had 72% deoxyribonucleic acid homology with the type strain of *C. gingivalis*. Deoxyribonucleic acid homology of four strains of *Bacteroides ochraceus* with the neotype strain of *C. ochracea* was 76 to 86%.

In 1956 Prévot et al. (9) described two strains of obligately anaerobic, gram-negative rods which were morphologically similar to *Fusiformis nucleatus* (Knorr) Prévot (*Fusobacterium nucleatum* Knorr). These strains differed from the other strains of *F. nucleatus* examined in Prévot's study in that they produced ochre-red-orange colonies in agar deeps, did not produce indole or a fetid odor, and were described as producing propionic, formic, and lactic acids. The strains were further characterized as fermenting carbohydrates to a low pH, reducing nitrates to nitrites, and not digesting gelatin, milk, or coagulated protein. The authors considered these two strains to be variants of *F. nucleatus* and named them *F. nucleatus* var. *ochraceus*. The strains, Pasteur Institute 1258B and 1411, had been isolated from purulent sputum and from a subcutaneous feline abscess caused by a bite from another cat, respectively.

In a later study from the Pasteur Institute, Sebald (11) examined the guanine plus cytosine content of many strains of anaerobic gram-negative rods maintained in the collection of the Service des Anaérobies and determined that *F. nucleatus* var. *ochraceus* (strain 1956C) had a guanine plus cytosine content of 42 mol% and that the guanine plus cytosine content of nine strains of *F. nucleatus* was 27.4 to 28.6 mol%. Because the base ratio of the strains of *F. nucleatus* var. *ochraceus* was similar to that which she found for species of *Ristella* (many members of which are now classified as *Bacteroides* in the 8th edition of *Bergey's Manual* [5]), Sebald proposed that *F. nucleatus* var. *ochraceus* be reclassified as *Ristella ochracea*.

*B. oralis* var. *elongatus* was described by Loesche et al. (6) in 1964 on the basis of a study of six strains isolated from the human oral cavity. Strains of *B. oralis* grew on the surface of blood agar plates incubated in an anaerobic atmosphere but not on the surface of blood agar plates incubated in an aerobic atmosphere. *B. oralis* var. *elongatus* was differentiated from *B. oralis* var. *oralis* principally by cellular morphology. The authors further noted that the morphology of *B. oralis* var. *elongatus* was suggestive of fusobacteria and that some oral strains resembling *B. oralis* var. *elongatus* have "probably been characterized as *Fusobacterium*." The strains were described as fermenting carbohydrates and forming acetic and succinic acids. Strain R42 was designated the type strain.

The type strain of *B. oralis* var. *elongatus*, the two strains on which Prévot et al. (9) based the original description of *F. nucleatus* var. *ochraceus*, and strain 1956C used by Sebald (11) are no longer extant. One strain of *B. oralis* var. *elongatus* (SS31, received from Loesche) and three strains of *R. ochracea* (Prévot 2017B, 2476B, and 2376A) were compared at the Virginia Polytechnic Institute Anaerobe Laboratory. The strains all appeared to belong to the same species. The characteristics of these strains conformed well to the original descriptions of the respective varieties and, in the case of the strains from the Pasteur Institute, to the characteristics observed at the Pasteur Institute. All strains grew well and produced yellowish colonies on blood agar plates incubated in a candle extinction jar (3, 4). This was not considered to be inconsistent with the original descriptions, because no information was given in previous descriptions (6, 9) about the ability of strains to grow in conditions of reduced oxygen tension or increased carbon dioxide concentration. All four strains produced succinic and acetic acids from glucose, as had been reported by Loesche et al. (6). Since fermentation acids reported by Prévot et al. (9) probably were analyzed by Duclaux

distillation, which is sometimes difficult to interpret, the acetic acid could have been thought to be propionic and formic acids. None of the four strains reduced nitrates to nitrites, as had been reported originally for *F. nucleatus* var. *ochraceus*. However, copies of original data sheets listing reactions observed at the Pasteur Institute also showed nitrate-negative reactions for these strains, indicating that this reaction was considered to vary within the species. Because of the similarity among the strains, *B. oralis* var. *elongatus* and *R. ochracea* were considered subjective synonyms, and a new combination, *Bacteroides ochraceus*, was proposed (4, 8).

In the early 1960s Elizabeth O. King, Center for Disease Control (CDC), differentiated a group of thin, gram-negative, saccharolytic rods which she designated the DF-1 group. Colonies of some strains were yellowish. On blood agar plates most strains grew better anaerobically or in the atmosphere of a candle extinction jar than they did in an aerobic atmosphere. The strains did not grow on MacConkey agar and were oxidase negative. They were described as fermentative, reducing nitrate, and not producing catalase or indole (12). The description of the DF-1 strains was similar to that of *B. ochraceus* (Table 1). The majority of the DF-1 strains were from human clinical specimens. It is highly probable that King did not recognize the close relationship between the DF-1 strains and *B. oralis* var. *elongatus* because the DF-1 strains were not obligately anaerobic, as *B. oralis* var. *elongatus* was presumed to be before 1972.

The genus *Capnocytophaga* has recently been proposed to include gliding, anaerobic to microaerophilic, gram-negative bacilli producing acetate and succinate as major metabolic end products and requiring carbon dioxide for both aerobic and anaerobic growth (S. C. Holt, E. R. Leadbetter, J. L. Simpson, E. D. Savitt, and S. S. Socransky, Arch. Microbiol., in press; E. R. Leadbetter, S. C. Holt, and S. S. Socransky, Arch. Microbiol., in press; S. S. Socransky, S. C. Holt, E. R. Leadbetter, A. C. R. Tanner, E. D. Savitt, and B. F. Hammond, Arch. Microbiol., in press; B. L. Williams and B. F. Hammond, Arch. Microbiol., in press). In previous deoxyribonucleic acid (DNA) homology studies by Williams and Hammond (in press), 27 of 28 strains of *Capnocytophaga* formed three mutually exclusive homology groups, represented by strains 25, 4, and 27. Because *B. ochraceus* ATCC 27872 and strain 25 had DNA sequence homology at the level of 77%, they were considered to belong to the same species, and the new combination *Capnocytophaga ochracea* was proposed (Socransky et al., in press). New species *C. sputigena* and *C. gingivalis* were proposed for the genotypic and phenotypic groups represented by strains 4 and 27, respectively. In this communication we report the results of DNA homology studies with additional reference strains of *B. ochraceus*, representative strains from CDC-designated DF-1, and the type or neotype strains of *C. ochracea*, *C. sputigena*, and *C. gingivalis*. Biochemical characteristics of the strains examined are also presented.

## MATERIALS AND METHODS

**Strains.** Sources of strains used in this study are presented in Table 2.

**DNA purification.** Cultures for DNA purification were grown in Todd-Hewitt broth with 0.5% yeast extract. Cells from 1.5 liters of culture were harvested during late-log growth phase, washed with 0.05 M tris(hydroxymethyl)aminomethane, 0.05 M ethylenediaminetetraacetic acid, and 0.1 M NaCl (pH 8.0) and resuspended in the same buffer containing 25% (wt/vol) sucrose. DNA was purified as described by Marmur (7) modified by the addition of 0.5 mg of pronase per ml (grade B, Calbiochem, San Diego, Calif.) during incubation with sodium lauryl sulfate. DNA was labeled in *Capnocytophaga* strains 25, 4, and 27 during growth in a medium containing amino acids, glucose, salts, and 5 $\mu$Ci of [$^3$H]thymidine (New England Nuclear Corp., Boston, Mass.). Labeled DNA was purified in the same manner as unlabeled DNA. The minimum specific activity achieved was 26,000 cpm/$\mu$g (*C. ochracea* strain 25). Purified DNA was dialyzed against distilled water and sheared by ultrasonic treatment to an approximate molecular weight of $2.5 \times 10^5$. All DNA preparations were stored frozen until used.

**DNA hybridizations.** DNA homo- and heteroduplex formations were assayed with the single-strand specific S1 endonuclease method of Crosa et al. (2). Hybridization mixtures of 150 $\mu$g of unlabeled DNA and 0.1 $\mu$g of $^3$H-labeled DNA were denatured at 100°C for 10 min in 1 ml of 0.42 M NaCl. Reassociation conditions were 55°C for 16 h ($T_m$ − 30°C for *C. ochracea* strain 25). Labeled DNA was similarly incubated in the absence of unlabeled DNA and with a known heterologous DNA (*Escherichia coli* K-12) to determine the levels of self-reassociation for each experiment. After incubation, duplicate portions (0.2 ml) were removed from the hybridization mixtures and subjected to 1,000 U of S1 endonuclease (Miles Laboratories, Inc., Elkhart, Ind.) for 20 min at 50°C. Duplicate portions were simultaneously incubated at 50°C but in the absence of the endonuclease. Radioactivity in double-stranded DNA segments was measured after termination of the S1 reaction and precipitation of DNA with cold 20% trichloroacetic acid (final concentration of 5%). The ratio of radioactivity in S1-treated and untreated portions was determined, and the results were normalized to the values of homologous reactions after correction for self-association of labeled DNA. Reported results are the average of at least three such hybridization experiments.

**Physiological observations.** Strains grown in bulk cultures for DNA purification were first tested

TABLE 1. *Characteristics described for F. nucleatus var. ocbraceus Prévot et al. 1956 (9), B. oralis var. elongatus Loesche et al. 1964 (6), R. ochracea (Prévot et al.) Sebald 1962 (11), B. ochraceus (Prévot et al.) Holdeman and Moore 1972 (4), C. ochracea Socransky et al. 1979 (Socransky et al., in press), C. gingivalis Socransky et al. 1979 (Socransky et al., in press), C. sputigena Socransky et al. 1979 (Socransky et al., in press), and CDC DF-1 group*[a]

| Characteristic | *F. nucleatus* var. *ochraceus* | *R. ochracea*[b] | *B. oralis* var. *elongatus* | *B. ochraceus* | *C. ochracea* | *C. gingivalis* | *C. sputigena* | DF-1 |
|---|---|---|---|---|---|---|---|---|
| No. strains | 2 | 9 | 6 | 5 | 27 | 25 | 6 | 107[c] |
| Acid from: | | | | | | | | |
| Arabinose | nr[d] | nr | −(+) | −(w) | − | − | − | − |
| Cellobiose | nr | nr | + | + | v | − | − | v |
| Fructose | nr | + | + | + | +(−) | −(+) | v | +(−) |
| Galactose | nr | + | + | + | +(−) | − | − | +(−) |
| Glucose | nr | + | + | + | + | + | + | + |
| Lactose | nr | + | +(−) | + | + | − | v | +(−) |
| Maltose | nr | + | + | v | + | + | + | +(−) |
| Mannitol | nr | nr | − | − | − | − | − | − |
| Mannose | nr | nr | + | + | + | + | + | + |
| Raffinose | nr | nr | + | + | +(−) | −(+) | −(+) | + |
| Rhamnose | nr | nr | − | −(w) | −(+) | − | − | − |
| Salicin | nr | nr | − | −(w) | −(+) | − | − | − |
| Sorbitol | nr | nr | − | − | − | − | − | − |
| Starch | nr | nr | + | + | + | − | v | +(−) |
| Sucrose | nr | + | + | + | + | + | + | + |
| Trehalose | nr | nr | − | − | − | − | − | v |
| Xylose | nr | nr | −(+) | −(w) | − | − | − | − |
| 10% bile growth | nr | nr | i | i | i | i | i | nt |
| $H_2S$ production | w[e] | + | −[f] | nr | − | − | − | −[g] |
| Indole production | − | − | − | − | − | − | − | − |
| Nitrate reduction | + | + | − | − | − | − | +(−) | +(−) |
| Gelatin digestion | − | − | − | w(−) | −(+) | −(+) | v | − |
| Milk digestion | − | − | nr | − | nr | nr | nr | − |
| Gas from glucose | − | − | − | − | nr | nr | nr | − |
| Products from glucose | PFL | FPL | AS | AS(l) | AS | AS | AS | AS[b] |
| G + C mol. % | nr | 43[i] | nr | nr | 37-41 | 38-40 | 33-37 | nt |

[a] Symbols: +, positive reaction for 90 to 100% of the strains; −, negative reaction for 90 to 100% of the strains; +(−), positive reaction for 60 to 90% of the strains; −(+), negative reaction for 60 to 90% of the strains; v, + reaction for 40 to 60% of strains; w, weak reaction; i, inhibited; nr, not reported; nt, not tested; A, acetic acid; F, formic acid; L, lactic acid; l, small amount of lactic acid; P, propionic acid; S, succinic acid.

[b] Reactions from Prévot et al. (10).

[c] Results from CDC.

[d] Although Prévot et al. (9) state that carbohydrates are strongly fermented with the production of acid but no gas, the specific carbohydrates fermented by this variety were not cited by Prévot until 1967 (10).

[e] In lead acetate medium.

[f] In peptone iron agar.

[g] In triple sugar iron agar.

[h] Results of seven strains.

[i] One strain, 1956C, tested by chromatographic separation (11).

for biochemical characteristics to ensure that DNA was purified from cultures of intended identities. Characterization methods described are those used in the laboratory of B. L. W. Since the biochemical characteristics of individual reference strains are not available in the literature, they are communicated here, together with the DNA homology data.

Preparation and inoculation of prereduced anaerobically sterilized (PRAS) basal peptone and yeast extract (PY) and PY with glucose (PYG) were as described by Holdeman et al. (3). The inocula for biochemical tests were taken from 3-day PY cultures. A 4-ml amount of inoculum was suspended in 100 ml of freshly prepared (non-PRAS) PY basal medium of which 2 ml was then aseptically dispensed into culture tubes previously autoclaved with 0.2 ml of 10× concentrated substrate in distilled water; 5 ml of diluted inoculum also was added to 5 ml of 2× gelatin medium (3). Final substrate concentrations were those listed in the *Anaerobe Laboratory Manual* (3). Cultures were incubated in an anaerobic atmosphere of 10% hydrogen, 10% carbon dioxide, and 80% nitrogen achieved by evacuation and replacement. Acid production from carbohydrates was measured with a pH meter after incubation at 35°C for 5 days. Assays for indole production, reduction of nitrate, and hydrolysis of gelatin were those described by Cowan (1). Metabolic end products were determined from PYG cultures as described by Holdeman et al. (3) with the Hewlett-Packard (Avondale, Pa.) model 5830A gas chromatograph equipped with a flame ionization detector and a Supelco (Supelco, Inc., Bellefonte, Pa.) column of 15% SP-1220, 1% $H_3PO_4$ on 100/120 Chromosorb WAW. Gliding motility was determined by dark-field microscopy of 24-h cultures in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 0.5% yeast extract (Difco Laboratories, Detroit, Mich.).

## RESULTS

**DNA sequence homology.** DNA hybridization experiments with labeled DNA from the type of neotype strains of *C. ochracea*, *C. sputigena*, and *C. gingivalis* are summarized in Table 3. Eleven of the twelve *B. ochraceus* and DF-1 preparations tested demonstrated significant reassociation of DNA sequences with those of the labeled DNA from *C. ochracea*. All four strains currently designated as *B. ochraceus* and two DF-1 strains showed more than 75% homology to *C. ochracea*. Five of the DF-1 strains were homologous to the *C. ochracea* reference DNA at an intermediate level of 62 to 71%. The *B. ochraceus* and DF-1 strains as well as *C. ochracea* 25 showed only a low level of homology with *C. sputigena* 4 (average of 19%) and even less with *C. gingivalis* 27 (average of 9%). DF-1 strain D6182 was homologous with *C. gingivalis* 27 at the intermediate level of 72%.

**Motility.** All strains demonstrated gliding motility when log-phase cultures were observed in dark-field microscopy. The rate of transloca-

TABLE 2. *Strains examined*

| No.[a] | Designation | Source |
|---|---|---|
| VPI 5569 | *B. ochraceus* | Prevot 2376C from actinomycosis (?) received as *Ristella ochracea* (*Fusiformis nucleatus* var. *ochraceus*). |
| VPI 5568 | *B. ochraceus* | Prevot 2467B from dental suppuration, received as *R. ochracea* (*F. nucleatus* var. *ochraceus*). |
| VPI 5567 | *B. ochraceus* | Prevot 2017B, received as *R. ochracea* (*F. nucleatus* var. *ochraceus*). |
| ATCC 27827 | *B. ochraceus* | VPI 2845 from Loesche SS31 from human oral cavity (*B. oralis* var. *elongatus*). |
| CDC A7846 | DF-1 | Colorado State Health Department M418 from Mercy Hospital, Denver, CO, from throat. |
| CDC B2375 | DF-1 | Washington State Health Department Mc90 from Stevens Memorial Hospital, Edmons, WA, from human throat. |
| CDC B2906 | DF-1 | California State Health Department 5323-1-69 from Sequoia Hospital, Redwood City, CA, from blood. |
| CDC B5473 | DF-1 | W. J. Martin, Mayo University, Rochester, MN, C1293 from human blood. |
| CDC C4295 | DF-1 | Florida State Health Department CDC 426 from University Hospital, Jacksonville, FL, from finger. |
| CDC C6100 | DF-1 | Wisconsin State Health Department 63313 from St. Mary's Hospital, Madison, WI, from human vaginal specimen. |
| CDC C7282 | DF-1 | California State Health Department 2053 from human submaxillary gland. |
| CDC D6182 | DF-1 | Colorado State Health Department 76-1680 from L. Barth Reller, University of Colorado Medical Center, Denver, from human blood. |
| 25 | *C. ochracea* | S. S. Socransky, Forsyth Dental Center, Boston, MA, from juvenile periodontitis. |
| 4 | *C. sputigena* | S. S. Socransky, Forsyth Dental Center, Boston, MA, from rapidly advancing adult periodontitis. |
| 27 | *C. gingivalis* | S. S. Socransky, Forsyth Dental Center, Boston, MA, from juvenile periodontitis. |

[a] VPI, Virginia Polytechnic Institute and State University; ATCC, American Type Culture Collection.

tion was 20 μm per min for the most actively motile culture tested (DF-1 strain B5473).

**Biochemical characteristics.** The results of tests for carbohydrate utilization assayed by acid production, production of indole, and hydrolysis of gelatin are listed in Table 3. Acid production was scored as positive if cultures with the test substrate had a pH of at least 0.5 pH unit below the PY medium culture. All strains used in this study produced acetic and succinic acids from PYG medium. Propionic and isovaleric acids were produced in low amounts (less than 1 milliequivalent per liter) by some strains.

## DISCUSSION

Anaerobic to microaerophilic, yellow- to orange-pigmented, gram-negative rods having features characteristic of organisms currently called

TABLE 3. *DNA homology results and biochemical characteristics of C. ochracea, C. sputigena, C. gingivalis, B. ochraceus, and CDC group DF-1*

| Characteristic | *C. ochracea* 25 | *B. ochraceus* | | | | CDC Group DF-1 | | | | | | | | *C. gingivalis* 27 | *C. sputigena* 4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VPI 5569 | VPI 5567 | ATCC 27872[a] | VPI 5568 | C4295 | C6100 | B5473 | B2906 | A7846 | B2375 | C7282 | D6182 | | |
| Percent hybridization with DNA from: | | | | | | | | | | | | | | | |
| *C. ochracea* 25 | 100 | 86 | 83 | 77 | 76 | 87 | 86 | 71 | 70 | 68 | 62 | 62 | 14 | 4 | 22 |
| *C. gingivalis* 27 | 14 | 12 | 8 | 6 | 7 | 8 | 11 | 8 | 12 | 9 | 11 | 7 | 72 | 100 | 11 |
| *C. sputigena* 4 | 23 | 19 | 18 | 22 | 15 | 18 | 22 | 9 | 19 | 18 | 23 | 23 | 7 | 9 | 100 |
| Acid from[b]: | | | | | | | | | | | | | | | |
| Amygdalin | + | + | + | + | + | + | + | + | (+) | − | − | − | − | − | + |
| Cellobiose | + | + | + | + | (+) | + | + | + | − | − | − | − | − | − | + |
| Esculin | + | (+) | + | + | + | + | + | + | + | − | − | − | − | − | + |
| Fructose | + | + | + | − | + | + | − | + | + | + | + | − | − | − | + |
| Galactose | + | (+) | + | + | + | + | + | + | + | + | − | + | + | − | + |
| Glycogen | + | + | + | + | (+) | + | + | − | − | − | + | + | − | − | − |
| Inulin | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + |
| Lactose | + | + | + | + | (+) | − | + | − | + | + | − | + | + | − | + |
| Mannose | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + |
| Raffinose | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + |
| Salicin | − | − | − | − | − | + | − | + | − | − | − | − | − | − | − |
| Starch | + | + | + | + | + | + | + | − | + | (+) | + | + | + | − | + |
| Nitrate reduction | − | − | − | − | − | − | − | + | + | + | + | + | − | − | − |
| Gelatin digestion | − | − | + | − | + | − | − | − | − | − | − | − | − | + | − |

All strains produced acid from glucose, maltose, and sucrose. No strain produced acid from arabinose, glycerol, mannitol, rhamnose, ribose, sorbitol, trehalose, or xylose and no strain produced indole.

[a] Homology data for *B. ochraceus* ATCC 27872 with the same labeled DNA preparations from *Capnocytophaga* strains 25, 27, and 4 has been previously reported (Williams and Hammond, in press), but is included here for the purpose of comparison.

[b] +, Drop in pH of greater than 0.5 pH unit as compared with the PY culture; (+), drop in pH of 0.3 to 0.5 pH unit as compared with the PY culture.

*B. ochraceus* or CDC group DF-1 are being isolated with increasing frequency from clinical specimens, particularly blood cultures and oral-facial lesions. These show a high degree of DNA homology with species of the recently described genus *Capnocytophaga*. Of the 12 strains of *B. ochraceus* and CDC group DF-1 examined in this study, 11 showed a high level of DNA homology with the neotype strain of *C. ochracea*, and one showed a high level of homology with the type strain of *C. gingivalis*.

Most strains showing significant homology to *C. ochracea* strain 25 also had a low level of sequence similarity with *C. sputigena* strain 4, as also has been observed with other strains of *C. ochracea*. The range of hybridization values of the DF-1 and *B. ochraceus* strains with *C. ochracea* 25 was similar to those observed among dental isolates of *C. ochracea*, but there was a proportionately larger number of DF-1 strains with lower hybridization values of 62 to 71% than had been observed with the dental strains. This is probably because the DF-1 strains chosen to include in this study reflected the extremes in phenotypic variation of strains that had been included in the group. This was done to try to establish the range of phenotypic variation that might occur among strains of the geno-species that would be determined by the homology studies.

The one DF-1 strain that showed a high level of DNA homology with *C. gingivalis* was a strain that fermented lactose, observed previously with only 8% of the strains studied. *C. gingivalis* strain D6182 also hydrolyzed starch and fermented galactose, which had not been reported previously for strains of this species (Socransky et al., in press).

None of the strains studied showed a high level of homology with *C. sputigena*. This is logical, assuming that the number of strains of these species reported by Socransky et al. (in press) is representative of the normal distribution of these species in the mouth. Of the strains studied by Socransky et al., only 10% (6 of 58) were *C. sputigena*. The distribution of the other two species, however, was nearly equal in their study, with 27 strains of *C. ochracea* and 25 strains of *C. gingivalis*.

The three species of *Capnocytophaga* are similar phenotypically. It appears that no single phenotypic test can be used to differentiate among them. In general, *C. ochracea* ferments the largest variety of carbohydrates tested and *C. gingivalis* the fewest (Socransky et al., in press). Phenotypic reactions of the three species have been reported previously (Socransky et al., in press).

## ACKNOWLEDGMENTS

The Virginia Polytechnic Institute portion of this research was supported by Public Health Service grant DE 05139 from the National Institute of Dental Research. DNA and phenotypic studies at the University of Washington were funded by Public Health Service grant DE 02600, National Institute of Dental Research. We also gratefully acknowledge the assistance of R. E. Weaver, J. G. Jordan, and J. J. Jorgensen.

## LITERATURE CITED

1. **Cowan, S. T.** 1974. Cowan and Steel's manual for the identification of medical bacteria. Cambridge University Press, London.
2. **Crosa, J. H., D. J. Brenner, and S. Falkow.** 1973. Use of a single-strand specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. J. Bacteriol. **115**:904–911.
3. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute Anaerobe Laboratory, Blacksburg, Va.
4. **Holdeman, L. V., and W. E. C. Moore.** 1972. *Bacteroides*, p. 27. *In* L. V. Holdeman and W. E. C. Moore (ed.), Anaerobe laboratory manual. Virginia Polytechnic Institute Anaerobe Laboratory, Blacksburg, Va.
5. **Holdeman, L. V., and W. E. C. Moore.** 1974. Genus I. *Bacteroides* Castellani and Chalmers 1919, 959, p. 385–404. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
6. **Loesche, W. J., S. S. Socransky, and R. J. Gibbons.** 1964. *Bacteroides oralis*, proposed new species isolated from the oral cavity of man. J. Bacteriol. **88**:1329–1337.
7. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **5**: 109–118.
8. **Moore, W. E. C., and L. V. Holdeman.** 1973. New names and combinations in the genera *Bacteroides* Castellani and Chalmers, *Fusobacterium* Knorr, *Eubacterium* Prévot, *Propionibacterium* Delwich and *Lactobacillus* Orla-Jensen. Int. J. Syst. Bacteriol. **23**:69–74.
9. **Prévot, A. R., P. Tardieux, L. Joubert, and F. de Cadore.** 1955. Recherches sur *Fusiformis nucleatus* (Knorr) et son pouvoir pathogène pour l'homme et les animaux. Ann. Inst. Pasteur (Paris) **91**:788–798.
10. **Prévot, A. R., A. Turpin, and P. Kaiser.** 1967. Les bactéries anaèrobies, p. 260–261. Dunod, Paris.
11. **Sebald, M.** 1962. Étude sur les bactéries anaèrobies gram-négatives asporulées. Imprimerie Barnéoud S. A., Laval.
12. **Weaver, R. E.** 1975. Guide to presumptive identification of non-*Enterobacteriaceae* gram negative fermentative rods. Center for Disease Control, Atlanta, Ga.