Determination of the Deoxyribonucleic Acid Composition and
Deoxyribonucleic Acid-Deoxyribonucleic Acid Hybridization
of Fusobacterium fusiforme, Fusobacterium polymorphum,
and Leptotrichia buccalis: Taxonomic Considerations

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The deoxyribonucleic acids (DNA) of Fusobacterium polymorphum ATCC 10953 and F. fusiforme ATCC 23726 were submitted to DNA-DNA hybridization procedures. A polynucleotide homology of 78% was demonstrated, indicating that the recent grouping of all oral Fusobacterium strains into one species, F. nucleatum, should be reevaluated. The polynucleotide homology between Leptotrichia buccalis ATCC 19616 and the two strains of Fusobacterium tested was less than 30%. The guanine plus cytosine (G+C) values of the DNA of Fusobacterium and Leptotrichia strains tested were similar and unusually low (24.5 to 25.6 mol%). Since bacteria of the family Bacteroidaceae reportedly have G+C values of 41 to 42 mol%, the placing of the genera Fusobacterium and Leptotrichia into this family is questionable. The similar unusually low G+C values of the DNA of Fusobacterium and Leptotrichia may indicate a close phylogenetic relationship for these genera.

Considerable confusion exists in the literature concerning oral strains of Fusobacterium. Knorr (14, 15) originated the generic name Fusobacterium and described three species based on cellular morphology: F. plautii-vincentii, F. polymorphum, and F. nucleatum. However, it became evident that the cellular morphology of the fusobacteria was too inconsistent to be a reliable taxonomic criterion. Several additional classification schemes were subsequently proposed that utilized colonial morphology and serological and biochemical characteristics (8, 24-26). Unfortunately, none of these characteristics was sufficient to allow subclassification of the oral fusobacteria. As a result, in 1941 Boe (1) proposed combining all oral fusobacteria into one species. More recently, a similar conclusion was reached by Werner and co-workers (27) when they also were unable to define consistent phenotypic criteria for the subclassification of these microorganisms. Thus, Moore and Holdeman (21) combined all oral fusobacteria into one species, F. nucleatum, the type species of Fusobacterium. In addition, the genus Fusobacterium was retained in the family Bacteroidaceae by Moore and Holdeman (21).

A second genus of oral bacteria, Leptotrichia, has often been confused with Fusobacterium because of morphological similarities (8, 25, 26). Boe (1) attempted to separate these genera, but the confusion continued when Hoffman (9) described L. buccalis as F. fusiforme. Moore and Holdeman (21) recognized the genus Leptotrichia as a separate entity and placed it in the family Bacteroidaceae. The present study was designed to investigate the taxonomic relationship between strains of Fusobacterium and of L. buccalis by comparing the guanine plus cytosine (G+C) contents of their deoxyribonucleic acids (DNA) and by using DNA-DNA hybridization. In addition, the placement of the genera Fusobacterium and Leptotrichia into the family Bacteroidaceae was evaluated by comparing the G+C content of their DNA to that reported for members of Bacteroides, the type genus of the family Bacteroidaceae.

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MATERIALS AND METHODS

Bacterial strains. The strains used in this study were F. fusiforme ATCC 23726, F. polymorphum ATCC 10953, L. buccalis ATCC 19616, and L. buccalis ATCC 23471. In Table 1 are listed the metabolic characteristics useful in distinguishing between Fusobacterium and Leptotrichia.

Cultivation. Cells were grown in a modified
tryptone medium consisting of 1% tryptone (Difco), 1% yeast extract (Difco), 0.125% K$_2$HPO$_4$, 0.125% MgSO$_4$·7H$_2$O, and 0.2% glucose, at pH 7.2 (7). Cultures were grown on a rotary shaker in GasPak anaerobic jars (BBL) at 37°C for 24 h and harvested by centrifugation at 10,000 × g for 10 min; the bacterial pellets were stored at −20°C until needed for DNA extraction.

**Isotope labeling.** DNA labeling was accomplished by adding 10 µCi of [1-³H]adenine (5 to 15 Ci/mmol; New England Nuclear Corp., Boston, Mass.) and [2-³H]thymidine (6.7 Ci/mmol; New England Nuclear Corp., Boston, Mass.) per ml to the above-mentioned medium. At least 1 mg/ml of unlabeled DNA was added, followed by scintillation counting. Percent contamination was determined by the procedures of Lowry et al. (17).

**DNA-DNA hybridization.** The hybridization procedure used was a slight modification of that described by Legault-Demare et al. (16) and later by DeLey and Tijtgat (5). Unlabeled DNA was denatured to single-stranded form by placing 50 ml of a 6× SSC solution containing 30 µg of DNA per ml into a flask and heating it in a 100°C water bath for 20 min. The flask was transferred to an ice bath, and 100 ml of cold 6× SSC was added quickly. Denaturation was monitored by comparing the absorbance at 260 nm of the undiluted DNA solution before and after heating. DNA was bound to nitrocellulose membrane filters (25 mm, 0.45-µm pore size, type B-6; Schleicher and Schuell, Inc., Germany) by passing it through 5 ml of the denatured DNA solution. Binding efficiency was determined by comparing the absorbance at 260 nm of the DNA solution before and after the passages through the filters. The filters were washed with 6× SSC and dried.

The dried filters were placed over high-intensity light, were re-dried, and end product was added. At least 5 µg of labeled DNA can be added to a filter with 30 µg of unlabeled DNA (1:6 ratio) without saturating the filter (5). However, in this study a minimum 1:35 ratio was used to insure a wide margin below the saturation level. To vials containing the dried filters was added 0.8 ml of 2× SSC-Mg$_2$SO$_4$ (1,403 g of NaCl, 0.796 g of Na$_2$ citrate, 50 ml of distilled water, and 30 ml of N$_2$-bubbled dimethyl sulfoxide [Me$_2$SO], after which 0.2 ml of labeled DNA (1 µg) was added and the vials were incubated in a 43°C water bath for 18 h (5, 16). Filters were then washed once in 30% Me$_2$SO$_4$-70% 2× SSC, once in 15% Me$_2$SO$_4$-85% 2× SSC, and three times in 2× SSC. After the filters were dried, scintillation fluid consisting of 4 g of Omnifluor (New England Nuclear Corp., Boston, Mass.) per liter of toluene was added, followed by scintillation counting. Percent hybridization was determined by dividing the counts per minute for the heterologous hybridization samples by the counts per minute for the homologous hybridization samples.

### Table 1. Metabolic characteristics useful in distinguishing between Leptotrichia and Fusobacterium

<table>
<thead>
<tr>
<th>Genus</th>
<th>H$_2$S production</th>
<th>Indole production</th>
<th>End product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptotrichia</td>
<td>-</td>
<td>-</td>
<td>Lactate</td>
</tr>
<tr>
<td>Fusobacterium</td>
<td>+</td>
<td>+</td>
<td>Butyrate</td>
</tr>
</tbody>
</table>

* Semisolid agar (BBL); 48-h growth.
* b Indole nitrate medium (BBL), 24-h growth, followed by the addition of Kovacs reagent.

can Potash and Chemical Co., W. Chicago, Ill.) was added to a final concentration of 1.251 g/ml. The gradients were centrifuged at 35°C for 45 h at 68,100 × g. Visible bands of DNA, detected when tubes were placed over high-intensity light, were recovered through the sides of the tubes. Labeled DNA was dialyzed with 2× SSC (0.3 M NaCl, 0.03 M Na$_2$ citrate, pH 7.0). Unlabeled DNA was dialyzed with 6× SSC (0.1 M NaCl, 0.09 M Na$_2$ citrate, pH 7.0). Quantitative measurements of DNA were by the method of Burton (4). The absence of protein contamination was monitored by the procedures of Lowry et al. (17).
RESULTS AND DISCUSSION

The G+C contents of the two strains of Fusobacterium and two strains of Leptotrichia tested, as well as those of other members of the family Bacteroidaceae, are shown in Table 2. The fusobacteria tested in this study, as well as those tested by Johnson (13), have similar, very low G+C values (25 to 27 mol%). The Leptotrichia strains also have similarly low G+C values. In contrast, the Bacteroides species have G+C values between 41 and 42 mol%, considerably higher than those for Fusobacterium and Leptotrichia strains.

The average binding efficiency of unlabeled DNA to nitrocellulose membrane filters was 92%. Only filters with at least 35 μg of DNA bound were used in these experiments.

The average efficiency of renaturation for these hybridization experiments was 47.3%.

Table 3 lists the DNA-DNA homology of the Fusobacterium and Leptotrichia strains tested. The DNA of each strain was labeled and tested against the unlabeled DNA of the other strain.

In the present study, F. fusiforme ATCC 23726 and F. polymorphum ATCC 10953 were found to have a 78% polynucleotide homology. DNA homology studies reported in the literature usually have revealed that strains of the same species have over 80% homology (2, 3, 6). On the other hand, bacteria of different species occasionally have been shown to have high homology levels ranging from 75% to as much as 94% (10, 12, 22, 23). The homology range of 75 to 85% seems to be the borderline for species differentiation. Because of the lack of definite guidelines for species differentiation, the combining of all oral fusobacteria into one species cannot be shown to be untenable on the basis of this study. However, at best, the DNA homology of the strains of F. polymorphum and F. fusiforme tested was at the lower end of the range acceptable for different strains of the same species. These results suggest that the combining of oral fusobacteria into one species should be reevaluated.

The polynucleotide homology between the two strains of L. buccalis and the two strains of Fusobacterium tested is less than 30%, too low to indicate more than the possibility of closely related genera.

The DNAs of the Leptotrichia and Fusobacterium strains studied have similar, very low G+C values (24.5 to 25.6 mol%). There is approximately a 16 mol% difference in the G+C content of the DNA of strains of these two genera and those of other members of the family Bacteroidaceae reported in the literature. It is unlikely that such large differences are due to variation in laboratory techniques, especially since one of the studies cited (13) reported G+C values for the Fusobacterium strains tested within 2% of the values reported here. It is concluded that Fusobacterium and Leptotrichia are not closely related to the Bacteroidaceae species reported and that these two genera probably do not belong in the family Bacteroidaceae. Furthermore, the similar, unusually low G+C values for Fusobacterium and Leptotrichia may indicate a close phylogenetic relationship for these genera.

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REPRINT REQUESTS

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LITERATURE CITED


