Actinomyces georgiae sp. nov., Actinomyces gerencseriae sp. nov., Designation of Two Genospecies of Actinomyces naeslundii, and Inclusion of A. naeslundii serotypes II and III and Actinomyces viscosus serotype II in A. naeslundii Genospecies 2

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DNA of type strains and representative members of Actinomyces groups from the human periodontal flora and from other habitats were compared by using the S1 nuclease procedure to determine their genetic relatedness. One rather common group from the human periodontal flora, previously called "Actinomyces D08," is phenotypically distinct from, and genetically unrelated to, previously described species. We propose the name Actinomyces georgiae for this organism; the type strain is strain ATCC 49285. Another common group from the human periodontal flora is Actinomyces israelii serotype II, which was found to be genetically distinct from the type strain of A. israelii (serotype I) and from other previously described species of Actinomyces. We propose the name Actinomyces gerencseriae for this organism; the type strain is strain ATCC 23860. A. naeslundii serotype I strains were distinct from the other strains studied. A separate genospecies which included strains of A. naeslundii serotypes II and III and A. viscosus serotype II was delineated. Strains of Actinomyces serotype WVA 963 constitute an additional distinct genospecies. Because there are no reliable phenotypic tests, other than serological analyses, to differentiate Actinomyces serotype WVA 963 and the two genospecies of A. naeslundii, no taxonomic changes are proposed for these three genospecies.

Actinomyces species and serotypes form a major portion of the periodontal flora of humans and animals. Although there is no direct evidence that these organisms cause gingivitis or periodontitis (26), they appear to play a key role in the colonization of gingival crevices that may relate to subsequent periodontal disease (18-22; P. K. Kolenbrander, Crit. Rev. Microbiol., in press). Precise identification of these taxa often is difficult because there is considerable phenotypic variation among strains within species and because specific antisera that are exceedingly helpful in species identification (9) are not available commercially.

Among the species isolated from humans, it generally has been accepted that Actinomyces israelii, Actinomyces odontolyticus, and Actinomyces meyeri are distinct species (31, 37). However, high degrees of phenotypic (8, 16, 33-35) and serological (5, 10, 14) relatedness between Actinomyces naeslundii and human isolates of Actinomyces viscosus have been reported previously. Gerencser (9) noted that "all recent evidence seems to support the earlier suggestions that A. naeslundii and A. viscosus are varieties of a single species," but she retained the current classification of considering catalase-negative strains A. naeslundii and catalase-positive strains A. viscosus, as did Schaal (31).

In a study of polyacrylamide gel electrophoresis patterns of whole-cell preparations from 22 strains of Actinomyces species, McCormick et al. (23) found that cluster analysis of polyacrylamide gel electrophoresis banding patterns permitted clustering first by serotype and then according to species. Two major divisions were reported. One contained strains of Actinomyces bovis serotype I, A. odontolyticus serotypes I and II, and A. israelii serotypes I and II. The other contained strains of A. naeslundii serotypes I, II, and III and A. viscosus serotypes I and II. Strains of Actinomyces serotype WVA 963, which previously had been described as A. naeslundii serotype IV (10), were not included in the study of McCormick et al.

Previous reports of DNA hybridization studies have been limited. Coykendall and Munzenmaier (6), who used a membrane filter hybridization analysis method, reported 71 to 107% DNA relatedness between A. naeslundii and human strains of A. viscosus. Despite these high levels of DNA relatedness, Coykendall and Munzenmaier did not propose combining the human strains of A. viscosus with A. naeslundii, based on the report by Fillery et al. (8) that the two groups could be separated phenotypically (with 12% dissimilarity). However, in discussing their own data, Fillery et al. (8) stated that "these data suggest that the division of human A. viscosus from A. naeslundii is closer to a serotypic separation than separation at a species level." Relationships among the other oral Actinomyces species were not reported by Coykendall and Munzenmaier.

Part of the problem of species delineation in the genus Actinomyces has been a dearth of information concerning the genetic relatedness among the many types found in the human flora. Such evidence is essential for reliable nomenclature and diagnosis. In this study we attempted to provide some of the needed information concerning the relationships of these taxa and in particular to determine the taxonomic position of serotype WVA 963 (= WVU 963) and strains that reacted with both A. naeslundii and A. viscosus serotype II antisera.

MATERIALS AND METHODS

Bacterial strains and characterization. The strains which we examined by using DNA hybridization and which were used in the preparation of fluorescent antibody conjugates are shown in Table 1. Additional strains for which phenotypic data were summarized were obtained from the Virginia Polytechnic Institute and State University Culture Collection and were isolated from dental plaque. These strains

* Corresponding author.
### TABLE 1. Strains used in this study

<table>
<thead>
<tr>
<th>Species or serotype</th>
<th>VPI no.</th>
<th>ATCC no.</th>
<th>Source and comments</th>
</tr>
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<tr>
<td>A. georgiae</td>
<td>D145A-07T&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49285&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Gingival crevice of healthy adult</td>
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<tr>
<td></td>
<td>D208G-6A&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Gingival crevice of young adult with rapidly progressive periodontitis</td>
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<td></td>
<td>D112H-02</td>
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<td>Gingival crevice of 14-yr-old</td>
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<td></td>
<td>D145A-07</td>
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<td>Gingival crevice of 11-yr-old</td>
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<td>A. naeslundii</td>
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<td>12104&lt;sup&gt;T&lt;/sup&gt;</td>
<td>CDC W826&lt;sup&gt;T&lt;/sup&gt;, human sinus</td>
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<td>W454, antigen strain for conjugate</td>
</tr>
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<td>49339&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>serotype II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>D163E-3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49340&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Gingival crevice of adult with progressive periodontitis</td>
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<td>49338&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>D133B-06</td>
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<td>D143E-06</td>
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<td></td>
<td>D153A-18B</td>
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<td>15987&lt;sup&gt;T&lt;/sup&gt;</td>
<td>CDC A828&lt;sup&gt;T&lt;/sup&gt;, naturally occurring periodontal disease in hamsters, antigen strain for <em>A. viscosus</em> serotype I conjugate</td>
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<tr>
<td>serotype I</td>
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<td></td>
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<td>12591&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27044</td>
<td>CDC W1053, human sputum, antigen strain for <em>A. viscosus</em> serotype II conjugate</td>
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<tr>
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</tr>
<tr>
<td>serotype NV</td>
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<td>Gingival crevice of juvenile with localized rapidly progressive periodontitis</td>
</tr>
<tr>
<td></td>
<td>D098C-06</td>
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<td>Gingival crevice of adult with naturally occurring periodontitis</td>
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<td></td>
<td>E055K-11</td>
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<td>Gingival crevice of 4-yr-old with gingivitis</td>
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<td>A. israelii</td>
<td>1966&lt;sup&gt;T&lt;/sup&gt;</td>
<td>12102&lt;sup&gt;T&lt;/sup&gt;</td>
<td>CDC W855&lt;sup&gt;T&lt;/sup&gt;, human brain abscess, antigen strain for <em>A. israelii</em> serotype I conjugate</td>
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<td>12594&lt;sup&gt;T&lt;/sup&gt;</td>
<td>23860&lt;sup&gt;T&lt;/sup&gt;</td>
<td>CDC W838&lt;sup&gt;T&lt;/sup&gt;, human parotid abscess, antigen strain for <em>A. israelii</em> serotype II conjugate</td>
</tr>
<tr>
<td>A. meyeri</td>
<td>8617&lt;sup&gt;T&lt;/sup&gt;</td>
<td>35568&lt;sup&gt;T&lt;/sup&gt;</td>
<td>A. R. Prevot (PIP 2477&lt;sup&gt;T&lt;/sup&gt;), human with purulent pleurisy</td>
</tr>
<tr>
<td></td>
<td>10648</td>
<td>33972</td>
<td>CDC W1148, antigen strain for conjugate</td>
</tr>
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<td>A. bovis serotype I</td>
<td>1965&lt;sup&gt;T&lt;/sup&gt;</td>
<td>13683&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lumpy jaw in cows, antigen strain for conjugate</td>
</tr>
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<td>A. bovis serotype II</td>
<td>12595</td>
<td></td>
<td>CDC WVU 292, antigen strain for conjugate</td>
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<td>1991-2&lt;sup&gt;T&lt;/sup&gt;</td>
<td>17929&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Deep carious lesions around teeth</td>
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<td>12589</td>
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<td>CDC W830, antigen strain for conjugate</td>
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<td>12590</td>
<td>Gingival crevice of healthy 12-yr-old</td>
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<td>CDC W1514 (= WVU 482), antigen strain for conjugate</td>
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<td>A. hordeoverylensis</td>
<td>14010&lt;sup&gt;T&lt;/sup&gt;</td>
<td>35275&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Canine ascites fluid</td>
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</tbody>
</table>

<sup>a</sup> All strains except the *A. georgiae*, *A. hordeoverylensis*, and *Actinomyces* serotype NV strains reacted 4+ with homologous conjugate and did not react with any of the other conjugates; strains of *Actinomyces* serotype NV reacted 3+ to 4+ with conjugates to *A. naeslundii* serotype I or II and *A. viscosus* serotype II and did not react with any of the other conjugates. Strains of *A. georgiae* and *A. hordeoverylensis* did not react with any of the conjugates. All strains except CDC WVU 820, WVU 963, VPI 10648, VPI 12595, and VPI 12589 were used in the DNA homology studies.

<sup>b</sup> VPI, Virginia Polytechnic Institute and State University, Blacksburg; ATCC, American Type Culture Collection, Rockville, Md.; CDC, Centers for Disease Control, Atlanta, Ga.; PIP, Pasteur Institute, Paris, France; WVU, West Virginia University, Morgantown.

<sup>c</sup> Strain used to prepare reference DNA.

<sup>d</sup> Strain that has been deposited with the American Type Culture Collection but has not been returned for depositor approval.
were limited to those for which cellular fatty acid data were available. For those species or serogroups for which multiple strains were available, each isolate was obtained from a different person and was characterized during the past 11 years. Thus, the phenotypic variation given below represents the variation obtained with multiple lots of media and reagents and should express the variation that would be seen within the species or serogroup.

Cultures were grown in prereduced anaerobically sterilized media and were characterized by using the procedures of Holdeman et al. (13) and Moore et al. (27). Unless otherwise specified, Tween 80 (0.02%, vol/vol) was added to all broth media. After isolation and purification, pure cultures were tested for maximum growth and fermentation in peptone-yeast extract-glucose broth medium with and without Tween 80 and were inoculated and incubated (i) anaerobically under CO2, (ii) aerobically and restoppered (to introduce a small amount of oxygen), and (iii) aerobically (tubes covered with sterile aluminum foil). Additional phenotypic tests were performed by using the inoculation and incubation conditions that produced the best growth and lowest pH. If all conditions produced the same results, the media used subsequently for characterization included Tween 80 and were inoculated aerobically and restoppered.

Cellular fatty acids were determined as previously described (25). Briefly, cells from 10-ml portions of peptone-yeast extract-glucose-Tween 80 cultures were harvested by centrifugation and washed once by suspension in 3 ml of 0.7% (wt/vol) aqueous MgSO4 and centrifugation. The cells were lysed and saponified with 1 ml of basic methanol (45 g of NaOH, 150 ml of methanol, 150 ml of distilled water), heated in a boiling water bath for 5 min, mixed with a vortex mixer, and heated in the boiling water bath for an additional 25 min. To methylate cell constituents, 1 ml of HCl-methanol (325 ml of 6.0 N HCl, 164 ml of methanol [certified grade]) and 1 ml of sulfuric acid-methanol (162.5 ml of H2SO4 [American Chemical Society reagent grade] added to 162.5 ml of distilled water, 275 ml of methanol [certified grade]) were added, and the solution was heated at 80°C for 10 min. After cooling, methylated components were extracted by adding 1.25 ml of hexane-ether (200 ml of hexane [high-performance liquid chromatography grade], 200 ml of methyl-tert butyl ether [high-performance liquid chromatography grade]) and shaking the tubes end over end for 10 min. The extract was washed once with 3 ml of a solution containing 5.4 g of NaOH in 450 ml of deionized distilled water saturated with NaCl. A 2-µl portion of the washed extract was chromatographed on a fused-silica capillary column in a model HP-5890A chromatograph (Hewlett-Packard Co., Palo Alto, Calif.) equipped with a flame ionization detector and a model HP-3392A integrator (Hewlett-Packard). We used the MIS software package (Hewlett-Packard) to identify the peaks (by retention time) and to determine the area, the ratio of area to height, the equivalent chain length, the total area, and the total area of named compounds; the MIS software package was also used to calculate the percentage of area for each named compound compared with the total area of named compounds. Identification was accomplished by using the Virginia Polytechnic Institute and State University Anaerobe Laboratory Broth Library and methods (Microbial ID, Inc., Newark, Del.).

Fluorescent antibody. Cells were serotyped with fluorescent antibody prepared by one of us (B.K.) by using previously described procedures (7, 11, 12), except that Sepharose 6B beads rather than Sepharose 6MB beads were used in the affinity column (7). The following antisera were used: A. israelii serotypes I and II; A. naeslundii serotypes I, II, and III; Actinomyces serotype WVA 963; A. viscosus serotypes I and II; A. meyeri; and A. odontolyticus serotypes I and II.

DNA isolation and hybridization. The organisms were grown in 300-ml volumes of a medium consisting of peptone-yeast extract-glucose (13) supplemented with 1% brain-heart infusion broth (Difco Laboratories, Detroit, Mich.) and 0.01% (vol/vol) Tween 80. Each flask was inoculated with 8 ml of culture grown in chopped meat broth-carbohydrate medium (13). The flasks were incubated at 37°C with stirring until there was heavy growth, usually about 40 h.

DNA was isolated by using a cetyltrimethylammonium bromide (CTAB) procedure which is a variation of a procedure that has been used to isolate fungal DNA (T. Flynn, personal communication). A harvested cell pellet (final weight, 10 g) was transferred to a tared Braun Disintegrator (Bronwill Scientific, Inc., Rochester, N.Y.) shaking bottle along with Tris-EDTA buffer (10 mM Tris hydrochloride, 1 mM EDTA, pH 8.0). Then 10 ml of 2× CTAB lysis buffer (1× CTAB lysis buffer is 50 mM Tris hydrochloride, 0.7 M NaCl, 10 mM EDTA, 1% [wt/vol] CTAB, and 1% [vol/vol] 2-mercaptopethanol, pH 8.0) and 20 ml of glass beads (diameter, 110 µm) were added to the bottle, and the bottle was shaken for 5 min with CO2 cooling. The lystate was separated from the glass beads by filtration through a coarse sintered glass filter. An additional 30 ml of 1× CTAB lysis buffer was used to wash the lysate from the beads quantitatively. The pooled lystate was digested with proteinase K (40 µg/ml) for 4 h at 60°C and then extracted with CHCl3. The cetyltrimethylammonium-nucleic acid salts were then precipitated by diluting the lystate with an equal volume of dilution-precipitation buffer (50 mM Tris hydrochloride, 10 mM EDTA, 1% [wt/vol] CTAB, pH 8.0). The precipitate was washed twice or three times with 40-ml volumes of 0.4 M NaCl to remove the free CTAB. The pellet precipitate was then dissolved in 8 ml of 2.5 M ammonium acetate, which was facilitated by warming in a 50°C water bath. The tube was then placed on ice, where much of the high-molecular-weight RNAs precipitated. After the precipitated RNAs were removed by centrifugation, the DNA and the remaining RNA were collected by ethanol precipitation. The pellet was dissolved in a solution containing 10 ml of 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate [pH 7.0]), 0.5 ml of RNase A (0.5 mg/ml), and 5 µl of RNase T1 (200 U/µl), and the mixture was incubated at 37°C for 1 h. The digest was then extracted once with phenol-CHCl3, adjusted to 0.3 M sodium acetate, and ethanol precipitated. The DNA preparations were dissolved in 0.1× SSC, and the purity of each preparation was estimated from the amount of hyperchromicity that occurred during its melting profile (17).

For the hybridization experiments, the DNA preparations were uniformly fragmented by three passages through a French pressure cell at 16,000 lb/in2. The preparations were then denatured by being heated in a boiling water bath for 5 min, and, after cooling in ice, the preparations were centrifuged at 12,000 × g for 15 min to remove any particulate material. The DNA concentration of each preparation was adjusted to 0.4 mg/ml. The preparations were stored at −20°C. Portions (2 to 3 µg) of the fragmented and denatured preparations from selected reference strains were labeled by iodination (36). The specific activities of the labeled preparations ranged from 1 × 108 to 2 × 108 cpm/µg of DNA. DNA sequence similarity values were determined by using the S1 nuclease procedure (17). Each of the reassociation vials contained 10 µl of labeled DNA (10 to 20 ng; 30,000
RESULTS AND DISCUSSION

The results of the DNA hybridization experiments and the guanine-plus-cytosine (G+C) contents of the strains which we examined are shown in Table 2. Strains of *A. israelii* serotype I, *A. meyeri*, *A. odontolyticus* serotypes I and II, *A. bovis*, and *Actinomyces hordeovulneris* were clearly distinct from the other species or serotypes.

The DNA hybridization data indicated that the organisms which we previously designated "Actinomyces D08" (28, 29) on the basis of the results of phenotypic tests, polyacrylamide gel electrophoresis patterns, and cellular fatty acid profiles and which were represented by reference DNAs from strains VPI D28G-6A and VPI D145A-7 (Table 2) have negligible DNA relatedness with the other reference DNAs tested and clearly represent a new species. Because these bacteria are facultatively anaerobic, nonsporing, nonmotile, gram-positive, filamentous to diphtheroid bacilli that produce combinations of lactic and succinic acids, usually along with acetic acid, 1.0 (range, 0.2 to 2.7). Small amounts of lactic acid (0.4 meq/100 ml; range, 0.1 to 0.8 meq/100 ml) are detected in 70% of the cultures, and pyruvate (0.6 meq/100 ml; range, 0.2 to 1.5 meq/100 ml) accumulates in 54% of the cultures. Lactate and pyruvate accumulate only in cultures that are inoculated aerobically and restoppered and are not detected in cultures that are inoculated under CO₂. Accumulation of lactate and pyruvate probably results from an insufficiency of bicarbonate in the medium or CO₂ in the headspace gas. No hydrogen is detected in the headspace gas of peptone-yeast extract-glucose-Tween 80 cultures incubated for 4 to 5 days.

Growth in prerduced anaerobically sterilized peptone-yeast extract-glucose-Tween 80 broth is turbid (85%) with smooth (38%), flaky (26%), crumbly or granular (24%), or stringy toropy (12%) sediment that occasionally adheres to the bottom of the tube. Broth cultures without turbidity often have crumbly to coarse granular sediment. Good growth is produced in peptone-yeast extract (basal medium) broth cultures, and excellent growth is produced in peptone-yeast extract medium that contains a fermentable carbohydrate. The pH values of peptone-yeast extract cultures that are inoculated aerobically and restoppered are 6.6 to 7.8; the pH values of peptone-yeast extract cultures that are inoculated under CO₂ are 5.7 to 6.3. The pH of peptone-yeast extract-glucose cultures is 4.7 (range, 4.5 to 5.2) and is the same under all conditions of inoculation if growth is equivalent. The acid products (in milliequivalents per 100 ml of culture) from 4- to 5-day-old peptone-yeast extract-glucose-Tween 80 broth cultures are as follows: succinic acid, 1.4 (range, 0.3 to 4.0); formic acid, 1.0 (range, 0.4 to 1.8); and acetic acid, 1.0 (range, 0.2 to 2.7). Small amounts of lactic acid (0.4 meq/100 ml; range, 0.1 to 0.8 meq/100 ml) are detected in 70% of the cultures, and pyruvate (0.6 meq/100 ml; range, 0.2 to 1.5 meq/100 ml) accumulates in 54% of the cultures. Lactate and pyruvate accumulate only in cultures that are inoculated aerobically and restoppered and are not detected in cultures that are inoculated under CO₂. Accumulation of lactate and pyruvate probably results from an insufficiency of bicarbonate in the medium or CO₂ in the headspace gas. No hydrogen is detected in the headspace gas of peptone-yeast extract-glucose-Tween 80 cultures incubated for 4 to 5 days.

Strains grow well at 37°C. No strain produces urease (urease broth) (13). Fermentation of glycerol is variable.

The major cellular fatty acids are 16:0, 18:1 cis-9, and 14:0.

There is no reaction with fluorescent antibody conjugates to *A. israelii* serotype I, *A. meyeri*, *A. odontolyticus* (Actinomyces gerencseriae [see below]), *A. naeslundii* serotypes I, II, and III, *Actinomyces* serotype WVA 963, *A. viscosus* serotype II, *A. meyeri*, and *A. odontolyticus* serotypes I and II.

Additional characteristics of the species are shown in Tables 3 and 4. Differential characteristics are shown in Table 5.

The type strain is strain VPI D145A-7 (= ATCC 49285).

The G+C content of the DNA ranges from 65 to 69 mol% (Table 1).

Isolated from human gingival crevices.

*A. georgiae* accounts for 1.4% of the human healthy periodontal flora (Table 6).

*A. israelii* serotype II strains. Initially, we were surprised at the low level of DNA relatedness between *A. israelii* serotype I reference DNA and unlabeled DNAs from *A. israelii* serotype II (strains VPI 12594 and VPI D140D-18). However, Stackebrandt and Charfreitag (39) recently reported that an *A. israelii* serotype I-specific DNA probe failed to give a hybridization signal with rRNA from a strain of *A. israelii* serotype II and suggested that the *A. israelii* serotype II strains may constitute a new species.

According to some numerical taxonomic analyses (16, 35), strains of *A. israelii* serotypes I and II cluster together.

Other workers (32, 33) have recognized four subgroups (subspecies) within *A. israelii*, with two of the subgroups corresponding to the two recognized serotypes. On the basis of the low levels of DNA-DNA relatedness with the other species tested (Table 2), we propose that *A. israelii* serotype II strains should be recognized as a distinct species, for which we propose the name *Actinomyces gerencseriae*.
TABLE 2. DNA relatedness of strains of Actinomyces species as determined by S, nuclease experiments

<table>
<thead>
<tr>
<th>Strain</th>
<th>A. odontotyticus</th>
<th>A. georgiae</th>
<th>A. naeslundii</th>
<th>A. israelii</th>
<th>A. meyeri</th>
<th>A. hordeovulneris</th>
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<tr>
<td>DNA content</td>
<td>45.0%</td>
<td>46.5%</td>
<td>45.0%</td>
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<td>Homology (%)</td>
<td>96.3%</td>
<td>95.7%</td>
<td>96.3%</td>
<td>95.7%</td>
<td>96.3%</td>
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<td>VPI 1401</td>
<td>VPI 1401</td>
<td>VPI 1401</td>
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</table>

*Actinomyces* species formerly was referred to as "Actinomyces DO8" (21, 22).

Actinomyces species II strains cross-react serologically with *A. naeslundii* and *A. viscosus* serotype I1 sera.

*Actinomyces* species I strains cross-react with *A. naeslundii* and *A. viscosus* serotype NV sera.

*Actinomyces* species I1 strains cross-react with *A. naeslundii* and *A. viscosus* serotype I1 sera.
Acid production from:
- Amygdalin: negative
- Arabinose: negative
- Cellobiose: negative
- Esculin: negative
- Esculin hydrolysis: positive

Acid production from:
- Fructose: negative
- Glucose: negative
- Glycogen: negative
- Inositol: negative
- Lactose: negative
- Maltose: negative
- Mannitol: negative
- Mannose: negative
- Melezitose: negative
- Melibiose: negative
- Raffinose: negative
- Rhamnose: negative
- Ribose: negative
- Salicin: negative
- Sorbitol: negative
- Starch: negative
- Starch hydrolysis: positive

Acid production from:
- Sucrose: negative
- Trehalose: negative
- Xylose: negative
- Gelatin hydrolysis (+w): negative
- Milk curd: positive
- Nitrate reduction: positive
- Catalase production: negative
- Hemolysin: negative

**TABLE 3. Characteristics of Actinomyces species and serotypes**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A. georgiae</th>
<th>A. naeslundii genospecies 1 (serotype I)</th>
<th>A. naeslundii genospecies 2*</th>
<th>Actinomyces serotype WVA 963</th>
<th>A. viscosus serotype I: reaction of 1 strain</th>
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<td>Acid production from:</td>
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<td>3</td>
<td>32</td>
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<tr>
<td>Esculin hydrolysis</td>
<td>$+$</td>
<td>89</td>
<td>95</td>
<td>100</td>
<td>+</td>
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</table>

| Acid production from: | | | | | |
| Fructose | $a^-$ | 94 | 95 | 97 | a |
| Glucose | a | 100 | 95 | 94 | a |
| Glycogen | aw | 91 | 4 | 45 | a |
| Inositol | $-a$ | 23 | 90 | 96 | a |
| Lactose | $a^-$ | 88 | 64 | 73 | w |
| Maltose | a | 97 | 95 | 98 | a |
| Mannitol | $-a$ | 31 | 2 | 6 | 0 |
| Mannose | $-a$ | 20 | 88 | 92 | a |
| Melezitose | $-a$ | 6 | 7 | 34 | a |
| Melibiose | $-a$ | 0 | 88 | 74 | a |
| Raffinose | $-a$ | 8 | 90 | 99 | a |
| Rhamnose | aw | 94 | 2 | 8 | 3 |
| Ribose | a | 97 | 48 | 74 | a |
| Salicin | $-a$ | 14 | 78 | 29 | a |
| Sorbitol | $-a$ | 11 | 7 | 24 | a |
| Starch | a | 97 | 28 | 72 | a |
| Starch hydrolysis | $+$ | 14 | 0 | 3 | 3 |

Acid production from:
- Sucrose: 100% positive
- Trehalose: 97% positive
- Xylose: 94% positive
- Gelatin hydrolysis (+w): 51% positive
- Milk curd: 97% positive
- Nitrate reduction: 17% positive
- Catalase production: 0% positive
- Hemolysin: 70% positive

**Notes:**
- a: A. naeslundii genospecies 2 contains A. naeslundii serotype II (8 strains tested), A. naeslundii serotype III (45 strains tested), A. viscosus serotype II (25 strains tested), and strains that react with both A. naeslundii and A. viscosus serotype II conjugates (serotype NV, 44 strains tested).
- c: +: Positive reaction for 95 to 100% of the strains; $-$: negative reaction or pH greater than 5.7 for 90 to 95% of the strains; $a$: pH 5.5 or less for 95 to 100% of the strains; v: pH 5.5 to 5.7 for 95 to 100% of the strains. Where two reactions are given, the first was the more common. The reactions for the type strains were the more common reactions except where indicated otherwise. The two reactions given account for all of the strains tested; for example, where the reactions were a,w and the percentage of positive strains was 95%, the remaining 5% produced only weak acid (pH 5.5 to 5.7). No strains digested meat, produced indole, or fermented erythritol.
- d: The reaction for the type strain was the less common reaction.

Other two subspecies of A. israelii recognized by Schaai and colleagues were not represented in our study because we examined only strains that reacted with the antisera available to us.

**Description of Actinomyces gerencseriae sp. nov.** Actinomyces gerencseriae (ge.ren.cse'ri.ae. N. L. gen. n. gerencseriae, of Gerencser, named in honor of Mary Ann Gerencser, an authority on Actinomyces species). Formerly, this taxon was called A. israelii serotype II, but genetically it is unrelated to the type strain of A. israelii. The DNAs of two strains of A. gerencseriae that were tested, strains VPI 12594 (= ATCC 23860) and VPI D140D-18, have reassocaiton values of 82%.

The description below is based on a study of 22 strains that reacted with the A. israelii serotype II antiserum and not with the antiserum of any of the other serotypes tested.

The type strain and 12% of the other strains tested are obligately anaerobic. The strains that grow on the surfaces of blood agar plates incubated in air enriched to 10% with CO₂ produce scant to moderately good growth. After incubation for 48 h, surface colonies of the type strain cultured anaerobically on blood agar plates are 0.2 mm in diameter, circular, peaked to pulvinate, lumpy, opaque, and white. Colonies of other strains are 1.0 to 2.0 mm in diameter (range, 0.2 to 2.0 mm), slightly irregular to circular, usually opaque (smaller colonies are transparent to translucent), lumpy (90%), and white (87%) or buff (13%).

Cells from 24-h peptone-yeast extract-glucose broth cultures are gram-positive, nonmotile, nonsporing, and usually filamentous with swellings in the cells. Branching cells are seen in some cultures.

Growth in prerduced anaerobically sterilized broth is crumbly (44%), stringy (22%), flaky (22%), or grainy (11%) and usually without turbidity (89%). Growth in peptone-yeast extract medium is moderate to good; excellent growth occurs in medium containing a fermentable carbohydrate. The pH of cultures in peptone-yeast extract-glucose broth cultures is 5.5 to 6.5, and the pH of cultures in peptone-yeast extract medium inoculated aerobically is 7.0 (range, 6.6 to 7.5). The pH in peptone-yeast extract-glucose-Tween 80 medium incubated for 3 to 5 days is 4.7 (range, 4.3 to 5.3). The acid products (in milliequiva-
lents per 100 ml of culture) of peptone-yeast extract-glucose-
TWEEN 80 cultures are as follows: lactic acid, 2.1 (range, 0.3
to 4.4); succinic acid, 1.0 (range, 0.2 to 3.0); formic acid, 0.7
(range, 0.2 to 2.0); and acetic acid, 0.6 (range, 0.1 to 1.8).
Pyruvate accumulates in about 20% of the cultures (average,
0.1 meq/100 ml of culture; range, 0.1 to 0.8 meq/100 ml in
those cultures that are positive). Similar ratios of products
are obtained under anaerobic or restoppered inoculation
conditions. No hydrogen is detected in the headspace gas.

Addition of TWEEN 80 to broth medium increases the
growth of 25% of the cultures and does not affect the growth
of 75%. No strain produces lecinthinase or lipase on egg yolk
agar or urease (urease broth method) (13). Glycerol is not
fermented by any of the 16 strains tested. The major cellular
fatty acids are 16:0 and 18:1 cis-9. Additional characteristics
of the species are shown in Tables 3 and 4. Differential
characteristics are shown in Table 5.

This species is distinct from A. israelii serotype I as
determined by polycrylamide gel electrophoresis banding
patterns (23), serological reactions, and the inability of strains of
A. gerencseriae to ferment 1-l-arabinose. Most (89%) of the strains of A. israelii serotype I ferment arabinose.

The type strain is strain CDC W838 (= VPI 12594 =
ATCC 23860), which was isolated from a human parotid
abscess.

The G+C content of the DNA is 70 to 71 mol%.
Isolated from human gingival crevices, mandibular ab-

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### Table 3—Continued

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<th>Reaction(s) of 26 strains</th>
<th>% of strains positive</th>
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viscosus (18 strains) led these authors to conclude that "the division of human A. viscosus from A. naeslundii is closer to a serotypic separation than separation at a species level." In another numerical taxonomic analysis of members of the family Actinomycetaceae, Schaal and Schaal (33) examined 40 to 50 strains of A. naeslundii, A. viscosus, and related organisms and reported that a number of discrete clusters are represented within a common cluster. As determined by using the Jaccard coefficient, with which clusters were defined at a similarity level of 55%, the within-group similarity ranged from about 65 to 75%, and the between-group similarity ranged from about 52 to 56%. The type strain of A. viscosus and six other animal strains clustered between the type strain and three other strains of A. naeslundii, A. viscosus, and related organisms and reported that a number of discrete clusters are represented within a common cluster. As determined by using the Jaccard coefficient, with which clusters were defined at a similarity level of 55%, the within-group similarity ranged from about 65 to 75%, and the between-group similarity ranged from about 52 to 56%. The type strain of A. viscosus and six other animal strains clustered between the type strain and three other strains of A. naeslundii and strains representing A. naeslundii serotypes II and III and some of the A. viscosus serotype II strains at a similarity level of about 60%. No taxonomic recommendations were made. Similar results, with higher similarity values as analyzed by using the simple matching coefficient, were reported by Schofield and Schaal (35), who observed that although the two species "are closely related and possibly should be combined," some phenotypic differences and the genetic evidence of Coykendall and Munzenmaier (6) suggest that "they are sufficiently distinct to merit separation, perhaps at the subspecies level." A similar position was maintained by Schaal in Bergey's Manual of Systematic Bacteriology (31).

Using the membrane filter method, Coykendall and Munzenmaier (6) found that the type strain of A. viscosus (serotype I) exhibited only 53 and 54% relatedness with reference DNAs from A. viscosus serotype II and A. naeslundii strains. We found a level of reciprocal relatedness of 36% between the type strains of A. viscosus and A. naeslundii by using the S1 nuclease method in this investigation. The lower value determined by the S1 nuclease method was expected and fits well with the comparative values previously reported when the same strains were examined by using different hybridization methods (2). In their comparison of reassociation values obtained when the same strains were examined by both membrane competition and S1 nuclease DNA-DNA relatedness methods, Bouvet and Grimont (2) showed that strains with 70% or greater reassociation values as determined by the membrane competition method gave S1 nuclease reassociation values of 35 to 50% (6 strains), 50 to 70% (13 strains), and >70% (8 strains). Indeed, it appears that some strains that would fit into a species cluster on the basis of membrane competition reassociation results have reassociation values as low as 33 to 34% when they are examined by using the S1 nuclease method. Therefore, on the basis of DNA reassociation values, there is reason to combine A. viscosus and A. naeslundii into a single species, albeit these taxa are at the low end of the acceptable range of relatedness for a genospecies. It also can be argued that the two taxa can be maintained as separate species, given the lower values reported by Coykendall and Munzenmaier (6) when a membrane filter method of hybridization was used. In a study of the intra- and intergeneric relationships of Actinomyces species as determined by comparisons of 16S rRNA sequences (39), the highest homology value reported was...
TABLE 4—Continued

% of total chromatographic area in:

<table>
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<th>A. viscosus</th>
<th>A. israelii</th>
<th>A. gerencseriae</th>
<th>A. bovis</th>
<th>A. odontolyticus</th>
<th>A. meyeri</th>
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</table>

95.4% between the type strains of A. naeslundii and A. viscosus. However, in the phylogenetic tree constructed by using a distance matrix method of Felsenstein that optimizes branch length, Stackebrandt and Charfreitag (39) found that A. viscosus branches between A. odontolyticus and A. bovis rather than with A. naeslundii and A. israelii. These authors explain this apparent anomaly by stating that "in the presence of varying evolutionary rates, species with the highest nucleic acid sequence similarity are not necessarily the most closely related ones" (phylogenetically). In light of this somewhat confusing situation, we do not propose that A. viscosus I and A. naeslundii be combined at this time.

However, our results (Table 7) clearly show that strains of A. naeslundii type II and strains of A. viscosus type II belong in the same species. Coykendall and Munzenmaier (6) previously reported that strain WVU 820 (our antigen strain for A. naeslundii type II antiserum) exhibited 85% DNA relatedness with DNA from the human A. viscosus strain which they tested. There also is a close relationship between these strains serologically. In the original description of A. naeslundii type II, Bragg et al. observed that there was extensive cross-reactivity between A. naeslundii type II and A. viscosus type II (S. L. Bragg, W. Kaplan, and G. Hageage, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, F6, p. 86). Obviously, not all strains share common antigenic determinants, but many do (e.g., the strains that we have referred to as Actinomyces serotype NV). The strains of Actinomyces serotype NV have average DNA relatedness values of 70 to 76% with reference DNAs from A. naeslundii type II and A. viscosus type II.

TABLE 5. Some differential characteristics of the Actinomyces species tested

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A. israelii</th>
<th>A. gerencseriae</th>
<th>A. naeslundii, A. viscosus serotype WVA 963&lt;sup&gt;a&lt;/sup&gt;</th>
<th>A. georgiace</th>
<th>A. bovis</th>
<th>A. odontolyticus</th>
<th>A. meyeri</th>
<th>A. hordeo-vulneris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduced</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Includes A. naeslundii genospecies I (serotype I), A. naeslundii genospecies 2 (A. naeslundii serotypes I and II), A. viscosus serotype II, and Actinomyces serotype NV.

<sup>b</sup> Positive reaction for 87 to 100% of the strains tested; -, negative reaction for 88 to 100% of the strains tested. Reactions for which no data are given are not differential.
strains (Table 7) and should be grouped with them. The strains of *A. naeslundii* serotype II, *A. viscosus* serotype II, and *Actinomyces* serotype NV exhibit average DNA relatedness values of 24 to 45% with the type strains of *A. naeslundii* and *A. viscosus* (Table 7) and should be considered a distinct species. However, other than by serological testing, there is no reliable way to differentiate strains of *A. naeslundii* serotype II and *A. viscosus* serotype II from strains of *A. naeslundii* serotype I (Tables 3 and 8). Therefore, we propose that these organisms should be retained in *A. naeslundii*, in keeping with the recommendation of Wayne et al. (41) that distinct genospecies “that cannot be differentiated from another genospecies on the basis of any known phenotypic property not be named until they can be differentiated by some phenotypic property.”

We found (Table 7) that strains of *A. naeslundii* serotype III are most closely related to strains of *A. naeslundii* serotype II, *A. viscosus* serotype II, and *Actinomyces* serotype NV (51 to 62%) and more distantly related to strains of *A. naeslundii* serotype I, *A. viscosus* serotype I, and *Actinomyces* serotype WVA 963 (20 to 37%). Reciprocal relatedness values of 51 and 62% between *A. naeslundii* serotypes II and III as determined by S1 nuclease hybridization (Table 7) correspond to a value of about 70% as determined by the membrane competition method (2), which indicates that *A. naeslundii* serotype III strains should be associated with the *A. naeslundii* serotype II-*A. viscosus* serotype II genospecies. Therefore, we propose that *A. naeslundii* genospecies 2 should contain strains currently belonging to *A. naeslundii* serotype II, *A. viscosus* serotype II, and *A. naeslundii* serotype III and the *Actinomyces* serotype NV strains. This grouping of *A. naeslundii* serotypes II and III in the same genospecies is consistent with the finding of Schaal and Schofield (33) that *A. naeslundii* serotypes II and III and some human isolates labeled *A. viscosus* form one of the phenotypic subclusters within the larger *A. naeslundii-A. viscosus* cluster.

*Actinomyces* serotype WVA 963 is more distantly related to the other strains tested (Tables 2 and 7). Gerencser and Slack (10) previously designated serotype WVA 963 as serotype IV of *A. naeslundii*, but that designation was not continued (9), possibly because workers were uncertain whether this serogroup represents a distinct species or is indeed a serotype of *A. naeslundii*. On the basis of numerical

---

**Table 7. Summary of DNA relatedness data for serotypes of *A. naeslundii* and *A. viscosus***

<table>
<thead>
<tr>
<th>Unlabeled DNA from:</th>
<th><em>A. naeslundii</em> serotype II</th>
<th><em>A. viscosus</em> serotype II</th>
<th><em>A. naeslundii</em> serotype NV</th>
<th><em>A. naeslundii</em> serotype III</th>
<th><em>A. viscosus</em> serotype I</th>
<th><em>Actinomyces</em> serotype WVA 963</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. naeslundii</em> serotype II</td>
<td>100</td>
<td>62</td>
<td>63</td>
<td>51</td>
<td>33</td>
<td>24</td>
</tr>
<tr>
<td><em>A. viscosus</em> serotype II</td>
<td>79</td>
<td>100</td>
<td>75</td>
<td>59</td>
<td>45</td>
<td>36</td>
</tr>
<tr>
<td><em>Actinomyces</em> serotype NV</td>
<td>76</td>
<td>70</td>
<td>100</td>
<td>52</td>
<td>41</td>
<td>32</td>
</tr>
<tr>
<td><em>A. naeslundii</em> serotype III</td>
<td>62</td>
<td>55</td>
<td>51</td>
<td>100</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td><em>A. naeslundii</em> serotype I</td>
<td>42</td>
<td>35</td>
<td>34</td>
<td>36</td>
<td>100</td>
<td>39</td>
</tr>
<tr>
<td><em>A. viscosus</em> serotype I</td>
<td>44</td>
<td>36</td>
<td>35</td>
<td>37</td>
<td>55</td>
<td>100</td>
</tr>
<tr>
<td><em>Actinomyces</em> serotype WVA 963</td>
<td>34</td>
<td>33</td>
<td>26</td>
<td>31</td>
<td>43</td>
<td>33</td>
</tr>
</tbody>
</table>

* Data were summarized from the data in Table 2. Values were determined by using the S1 nuclease method.

* Actinomyces strains that react serologically with *A. viscosus* serotype II and *A. naeslundii* serotype I or II antisera.

* Values are the averages of the values shown in Table 2.
analyses, Schaal and Schofield (34) reported that Actinomyces serotype WVA 963 clustered on the periphery of the A. israelii cluster. In later reports, Schaal and Schofield (33) and Fillery et al. (personal communication cited by Schaal and Schofield) found that strain WVA 963 was more closely related to the A. naeslundii-A. viscosus cluster. Our data indicate that strains of Actinomyces serotype WVA 963 represent a distinct genospecies that is closely related to A. naeslundii and A. viscosus. However, because these organisms cannot be differentiated phenotypically (Table 3), we are not proposing a change in their nomenclatural status. Genospecies Actinomyces serotype WVA 963 constitutes 3.4% of the bacterial flora of healthy gingival crevices (Table 6).

Phenotypic reactions of the species and serotypes which we studied are shown in Tables 3 and 8, and differential characteristics (at the 87% level of sensitivity) are shown in Table 5. Because A. viscosus, strains of Actinomyces serotype WVA 963, and the A. naeslundii genospecies cannot be differentiated from each other phenotypically, they are grouped together in Table 5.

The cellular fatty acids of A. viscosus, the genospecies of A. naeslundii, and Actinomyces serotype WVA 963 are shown in Table 4. All of these organisms have major amounts of 18:1 cis-9 and 16:0 fatty acids. In the percentage of 16:0 FAME detected, the one strain of A. viscosus serotype I which we tested is more similar to A. bovis, A. odontolyticus, A. meyeri, and A. horodevulnaris than to the genospecies of A. naeslundii and strains of Actinomyces serotype WVA 963. However, limited weight should be given to this observation insasmuch as only one strain of A. viscosus has been tested.

It is possible that the low levels of 14:1 cis-9 FAME, 18:2 cis-9,12 FAME, and 18:1 cis-9 dimethylacetyl may prove to be useful in differentiating A. viscosus serotype I and Actinomyces serotype WVA 963 from A. naeslundii. However, because these fatty acids constitute 1% or less of the fatty acids detected, species or serotype differentiation on the basis of their presence or absence would be tenuous. The cellular fatty acid profiles which we found for A. naeslundii, A. viscosus, and Actinomyces serotype WVA 963 are similar to those reported by Wada et al. (40) for A. viscosus NY1. However, we did not observe the branched-chain cellular fatty acids in cultures of A. naeslundii ATCC 12104T (serotype I) and A. viscosus ATCC 15987T reported by Wada et al. Even though our cells were grown in medium containing Tween 80, it is doubtful that the presence of Tween 80 in the medium accounted for this much difference in cellular fatty acid composition, which we cannot explain.

Other species of the genus Actinomyces. Although there have been reports, no verified strain of A. bovis (normally from lumpy jaw of cattle) has been found in human infec-
tions. However, we recently received a strain of *A. bovis* isolated from a llama. The characteristics of the strains of *A. bovis* which we examined are shown in Tables 3 and 4.

The DNA reassociation values for *A. odontolyticus* serotype II strains with *A. odontolyticus* serotype I reference DNA are 41 to 51% as determined by the S1 nuclelease method. This range is within the lower limit of acceptable reassociation values at the species level, so no taxonomic changes are recommended. In addition to succinic, lactic, acetic, and formic acids, pyruvic acid accumulates in many *A. odontolyticus* peptone-yeast extract-glucose-Tween 80 cultures (pH 4.8) that are inoculated aerobically and restopped, probably because of the decreased amount of CO₂ in the headspace gas. Tween 80 enhanced the growth and fermentation of most strains and inhibited none. Broth growth is turbid (97%) with smooth (53%), grainy (30%), or stringy toropy (17%) sediment. Surface colonies are circular, entire, convex (77%), pulvinate (19%), or flat (4%), smooth, and shiny. We found that the typical red colonies described for *A. odontolyticus* could be demonstrated in only 58% of the serotype I strains and 43% of the serotype II strains. The major cellular fatty acids (Table 4) are 18:1 cis-9, 16:0, and 14:0. Other characteristics of the species and serotypes are shown in Table 3.

*A. hordeoavulnertis*, which was isolated from dogs (4), was included in this study because it is phenotypically similar to *A. meyeri* (from human periodontal flora and brain abscesses) and has similar electrophoretic patterns of soluble cellular proteins. However, we found that these two species do not exhibit genetic relatedness (Table 2). The major cellular fatty acids of *A. hordeoavulnertis* are 18:1 cis-9, 16:0, and 14:0. Other characteristics of this species are shown in Tables 3 and 4.

There is considerable variation in phenotypic reactions among strains of different *Actinomyces* species. As pointed out by Slack and Gerencser (37), cultural conditions may affect the percentage of cultures that ferment certain carbohydrates. We routinely add Tween 80 to media for *Actinomyces* species, and for many we purposely admit some air into the stoppered tubes of prereduced media to improve growth. We suspect that one problem in obtaining uniform results is that the inoculum often is a lumpy or crumby suspension of cells. Test media may not receive uniform amounts of actual cellular inoculum as it is dispensed from Pasteur pipettes. However, we did observe very few cross-reactions among the distinct species revealed by DNA homology data, except for the reaction of the *Actinomyces* serotype NV strains with both *A. viscosus* serotype II and *A. naeslundii* antisera. These strains usually reacted with *A. naeslundii* serotype II or *A. naeslundii* serotype I antisera and rarely reacted with *A. naeslundii* serotype III antisera. When the organisms were originally tested, the reactions with the *A. naeslundii* antisera were equal to those of the control. However, in recent tests with different lots of antisera, the fluorescence with the *A. viscosus* serotype II conjugate was equal to that of the control antigen, but the fluorescence with *A. naeslundii* conjugates was much weaker than that originally observed.

It continues to be exceedingly difficult to differentiate among the described species of *Actinomyces* by usual phenotypic tests, although a few tests are helpful. We confirmed the report of other authors that L-arabinose fermentation best distinguishes *A. gerencseriae* (previously *A. israelii* serotype II) from *A. israelii* serotype I. Our finding that 89% of *A. israelii* serotype I strains ferment L-arabinose is within the range of 71 to 100% reported previously (3, 38), and we confirmed the previous reports that *A. gerencseriae* strains are negative for L-arabinose fermentation.

The cellular fatty acid constituents of the *Actinomyces* species and serotypes of *Actinomyces* species grown at 37°C in peptone-yeast extract-glucose-Tween 80 broth shown in Table 4 are in general agreement with those cited previously (1), except that we did not observe the large amounts of 18:0 FAME reported by Amdurr et al. (1).

The Microbial Identification System (Microbial Indentification System, Dover, Del.), which is based on a principal-component analysis of the relative amounts of these compounds, currently correctly identifies new isolates of these genospecies (as first choice) with 66 to 100% accuracy (Table 9). The accuracy of identification of serotypes within *A. naeslundii* genospecies 2 is only 37 to 65% (first choice) or 55 to 81% (first and second choice). The accuracy is improved when all serotypes of the genospecies are considered together because the first or second choice usually is a serotype included in *A. naeslundii* genospecies 2.

The type strain of *A. odontolyticus* (strain ATCC 17929), which is a serotype I strain, consistently comes out *A. odontolyticus* serotype II as the first choice and *A. odontolyticus* serotype I as the second choice. Similarly, the type strain (and antigen strain) of *A. gerencseriae* is identified as *A. israelii* serotype I (first choice) or *A. gerencseriae* (second, third, or fourth choice). As more verified strains of each taxon are used to calculate the mean reference pattern, the accuracy of the identification method can be expected to improve. The numbers of strains of *A. bovis* and *A. viscosus* serotype I available to us are too small to make an estimate of the accuracy of identification based on the patterns of the constituents.

Although it appears to be a minor problem with absorbed antisera, as pointed out by Slack and Gerencser (37), there are some cross-reactions among *A. naeslundii*, *A. israelii*, *A. gerencseriae*, and *A. odontolyticus* serotype II. Therefore, unless the reference patterns are based entirely upon many more DNA homology strains, the accuracy may not increase greatly. As a practical matter, we currently must rely on the strongest fluorescent reactions compared with the reactions of the known reference strains. The multiple strains of *A. georgiae*, *A. gerencseriae*, *Actinomyces* serotype WVA 963, and *Actinomyces* serotype NV were selected for DNA analysis on the basis of both serological and cellular fatty acid results, and they grouped together in DNA-related groups as predicted by those analyses (Table 2).

Among the isolates of *Actinomyces* species from gingival crevices (Table 6), there are numerous additional phenotypically distinct isolates, as well as isolates that have the phenotypic characteristics of one of the taxa described above but do not react with any of the available antisera.
TABLE 9. Agreement between cellular fatty acid identifications and identifications made by using fluorescent antibody or phenotypic tests

<table>
<thead>
<tr>
<th>Species or serotype</th>
<th>Identified bya</th>
<th>Total no. of analysesb</th>
<th>% of correct cellular fatty acid identifications ac</th>
<th>Most common other second choice(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FA, PT</td>
<td></td>
<td>First choice</td>
<td>First and second choices</td>
</tr>
<tr>
<td>A. bovis serotype I</td>
<td>FA</td>
<td>3</td>
<td>100</td>
<td>100 A. naeslundii serotype I</td>
</tr>
<tr>
<td>A. bovis serotype II</td>
<td>FA</td>
<td>3</td>
<td>100</td>
<td>100 “Actinomyces D20”</td>
</tr>
<tr>
<td>A. georgiae</td>
<td>FA</td>
<td>143</td>
<td>90</td>
<td>90 “Bifidobacterium D02A”</td>
</tr>
<tr>
<td>A. gerencseriae</td>
<td>FA</td>
<td>119</td>
<td>93</td>
<td>93 A. israelii serotype I, A. naeslundii serotypes I and II</td>
</tr>
<tr>
<td>A. israelii serotype I</td>
<td>FA</td>
<td>64</td>
<td>78</td>
<td>78 A. gerencseriae and Bifidobacterium infantis</td>
</tr>
<tr>
<td>A. meyeri</td>
<td>FA</td>
<td>36</td>
<td>92</td>
<td>92 A. georgiae, A. odontolyticus serotypes I and II</td>
</tr>
<tr>
<td>A. naeslundii genospecies 1 (serotype I)</td>
<td>FA</td>
<td>246</td>
<td>66</td>
<td>66 Serotype WVA 963, A. gerencseriae, A. naeslundii serotype III</td>
</tr>
<tr>
<td>A. naeslundii genospecies 2</td>
<td>Serotype II</td>
<td>FA</td>
<td>27</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Serotype III</td>
<td>FA</td>
<td>157</td>
<td>65</td>
</tr>
<tr>
<td>A. viscosus serotype II</td>
<td>FA</td>
<td>69</td>
<td>48</td>
<td>48 Actinomyces serotype NV, A. naeslundii serotype II</td>
</tr>
<tr>
<td>Actinomyces serotype NV</td>
<td>FA</td>
<td>126</td>
<td>62</td>
<td>62 A. naeslundii serotype I, A. israelii serotype I</td>
</tr>
<tr>
<td>All genospecies 2 strains</td>
<td>FA</td>
<td>379</td>
<td>79</td>
<td>79 A. naeslundii serotype I, A. israelii serotype I</td>
</tr>
<tr>
<td>A. odontolyticus serotype I</td>
<td>FA</td>
<td>88</td>
<td>73</td>
<td>91 A. odontolyticus serotype II</td>
</tr>
<tr>
<td>A. odontolyticus serotype II</td>
<td>FA</td>
<td>20</td>
<td>90</td>
<td>100 A. odontolyticus serotype I, A. meyeri</td>
</tr>
<tr>
<td>A. viscosus serotype I</td>
<td>FA</td>
<td>13</td>
<td>100</td>
<td>100 “Actinomyces D01”</td>
</tr>
<tr>
<td>Actinomyces serotype WVA 963</td>
<td>FA</td>
<td>129</td>
<td>74</td>
<td>83 A. naeslundii serotype I, A. israelii serotype I</td>
</tr>
</tbody>
</table>

a FA, Fluorescent antibody method; PT, phenotypic test results.
b Includes additional strains not used for reference patterns (Table 5).
c Percentage of correct identifications made by cellular fatty acid analysis compared with other serotypes or species of the genera Actinomyces (15 taxa), Bifidobacterium (15 taxa), and Lactobacillus (65 taxa).

Together, these isolates accounted for 7.6% of the total healthy flora and 5.4% of the flora of people with gingivitis or periodontitis (26, 28). Thus, Actinomyces species accounted for 25 to 30% of the bacterial isolates from human gingival crevices (26, 28). They are associated with early colonization of the teeth and gingival crevices, are known to attach to other bacteria (19, 21), and therefore may influence or determine the subsequent composition of the periodontal flora.

ACKNOWLEDGMENTS

We gratefully acknowledge the microbiological assistance of Sue C. Smith, Dianne M. Wall, and Kathy H. Pennington. We are indebted to Thomas O. MacAdoo, Virginia Polytechnic Institute and State University, for confirmation of specific epithets and their derivations and to Mary Ann Gerencser, J.-S. Chen, and D. Dean for critical reviews of the manuscript.

This work was supported by Public Health Service grants DE-05139 and DE-05054 from the National Institute of Dental Research, by grant 88-34116-3790 from the U. S. Department of Agriculture, and by project 131052 from the Commonwealth of Virginia.

LITERATURE CITED


