Recognition of Biovar C of *Fusobacterium necrophorum* (Flügge) Moore and Holdeman as *Fusobacterium pseudonecrophorum* sp. nov., nom. rev. (ex Prévot 1940)

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The cellular morphology, colonial morphology, biochemical properties, DNA base compositions, and DNA-DNA homologies of three biovars of *Fusobacterium necrophorum* were examined. Some differences were found among the three biovars in cellular morphology, colonial morphology, and biochemical properties. The guanine-plus-cytosine contents of DNAs from biovar C strains Fn521T (T = type strain), Fn522, and Fn520 were 30.4, 29.3, and 28.0 mol%, respectively, and the guanine-plus-cytosine contents of DNAs from strains VPI 2891 (biovar A) and VPI 6161 (biovar B) were 31.3 and 32.0 mol%, respectively. Labeled DNA from biovar C strain Fn521T exhibited 96 and 82% relatedness to DNAs from biovar C strains Fn522 and Fn520, respectively; however, it exhibited only about 10% relatedness to DNAs from strains of biovars A and B. Labeled DNAs from strains VPI 2891 and VPI 6161 exhibited more than 70% relatedness to each other, but about 6 to 20% relatedness to DNAs from biovar C strains. Therefore, *Fusobacterium pseudonecrophorum* sp. nov., nom. rev. (ex Prévot 1940) is proposed for *Fusobacterium necrophorum* biovar C. The type strain is strain Fn521 (=JCM 3722).

*Fusobacterium necrophorum* was previously classified in three distinct species, "*Sphaerophorus necrophorus*," "*Sphaerophorus funduliformis*," and "*Sphaerophorus pseudonecrophorus*" (17); later, *F. necrophorum* was divided into three biovars (biovars A, B, and C, respectively) (8). Although the three biovars are similar with respect to biochemical test results, biovar A, corresponding to "*S. necrophorus*," produces hemagglutinin and hemolysin; biovar B, corresponding to "*S. funduliformis*," produces hemolysin but no hemagglutinin; and biovar C, corresponding to "*S. pseudonecrophorus*," produces neither hemagglutinin nor hemolysin. Recently, it was reported that biovars A and B are sensitive to bacteriophage Fnpl, but biovar C is not sensitive to this bacteriophage (22). Biovar C strains are not pathogenic for mice, in contrast to strains of biovars A and B (8).

In this study, we observed cellular morphology and colonial morphology, examined biochemical properties, and determined levels of genetic relatedness by measuring DNA base compositions and degrees of DNA-DNA hybridization of strains of these three biovars.

**MATERIALS AND METHODS**

**Bacterial strains and media.** The following strains were studied: three strains of biovar C, strains Fn521T (strain 118T of H. Beerens, Institut Pasteur de Lille, Lille, France; an ovine pulmonary abscess isolate) (T = type strain), Fn522 (strain 130 of H. Beerens; an ovine pus isolate), and Fn520 (strain 410 of H. Beerens; a bovine feces isolate); biovar A, strain VPI 2891 (Flévez strain 2358; the parent strain of strain ATCC 25286); and biovar B strain VPI 6161 (a human spinal fluid isolate). The bacterial strains were subcultured in beef extract-peptone-Phytone-yeast extract extract medium (14). Plates were incubated anaerobically by using the steel wool method (3), with the air replaced with CO₂ at 37°C for 72 h.

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The bacterial strains were preliminarily grown once or twice in beef extract-peptone-yeast extract medium (21) and used for the tests described below.

**Biochemical tests.** The media and reaction conditions used for biochemical tests have been described previously (20).

**Preparation of DNA.** The procedure used for the extraction and purification of DNA was the hydroxyapatite batch method of Britten et al. (5).

**Determination of the G+C contents of DNAs.** The guanine-plus-cytosine (G+C) contents of the DNAs were determined optically by the thermal denaturation methods of Marmur and Doty (13), using a model UV190 automatic recording spectrophotometer (Shimazu, Tokyo, Japan). DNAs from calf thymus (G+C content, 42 mol%) and *Escherichia coli* K-12 (G+C content, 51 mol%) were used as references. Tests were carried out at least three times.

**Procedure for labeling DNA.** Purified native DNAs from strains Fn521T, VPI 2891, and VPI 6161 were labeled by using the nick translation method (12, 18).

**DNA-DNA homology experiments.** Hybridization tests were carried out by using the method of Aulakh and Gallo (2), with partial modification (9). The hybridization mixture contained 260 μg of unlabeled DNA per ml and 0.25 μg of labeled DNA per ml in a final volume of 100 μl of 0.48 M phosphate buffer containing sodium dodecyl sulfate. After the mixture was denatured at 105°C for 10 min, it was incubated at 63°C for 24 h. The C₅₀ value was about 420 mol · liter⁻¹. After incubation the hybrids were processed on a hydroxyapatite column preequilibrated at 60°C with 0.12 M phosphate buffer containing 0.2% sodium dodecyl sulfate.

**RESULTS AND DISCUSSION**

**Cellular morphology and colonial morphology.** After 3 days of incubation, colonies of biovar C strains on GAM agar (Nissui Seiyaku, Tokyo, Japan) supplemented with 5% defibrinated horse blood were about 2.5 mm in diameter, umbonate, grayish, and translucent. The margins were undulate, and the surfaces were granular. The cells were thick, semilong rods and filamentous. Colonies of strain VPI 2891
were about 2 mm in diameter, flat, grayish, and translucent. The margins were irregular, and the surfaces were matte and granular. The cells were thin, variable in length, and some-
times filamentous. Colonies of strain VPI 6161 were about 1
mm in diameter, conical, grayish, and opaque. The margins
were entire, and the surfaces were smooth. The cells from
colonies on GAM agar were thin; they looked like chains of cocci or short rods.

Biochemical and biological characteristics. Results of bio-
chemical and biological tests for the five strains are shown in
Table 1. All of the strains produced butyric acid from
glucose and propionic acid from lactic acid, reduced nitrate to nitrite, produced catalase, liquefied gelatin, grew in 20% bile, or produced acid from amygdalin, arabinose, cellobiose, dextrin, galactose,
glycerogen, inulin, lactose, maltose, mannitol, mannose, melibiose, raffinose, salicin, starch, sucrose, trehalose, or xylitol.

| Species                      | Strain          | Lipase reaction | Hemo-
lysis | Hemagglutina-
tion | Growth in liquid medium | Pathogenicity for mice | Acid produced from: |
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<tr>
<td>F. pseudonecrophorum</td>
<td>Fn521T</td>
<td>-</td>
<td>&lt;1:1</td>
<td>-</td>
<td>Turbid</td>
<td>No liver abscess formation</td>
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<tr>
<td></td>
<td>Fn522</td>
<td>-</td>
<td>&lt;1:1</td>
<td>-</td>
<td>Turbid</td>
<td>No liver abscess formation</td>
</tr>
<tr>
<td>F. necrophorum</td>
<td>VPI 2891</td>
<td>+</td>
<td>β</td>
<td>1:128</td>
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<td>Liver abscess formation (lethal)</td>
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<tr>
<td></td>
<td>VPI 6161</td>
<td>+</td>
<td>β</td>
<td>1:4</td>
<td>Sedimentary</td>
<td>Liver abscess formation (nonlethal)</td>
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| Species                      | Strain          | Lipase reaction | Hemo-
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<td>Liver abscess formation (nonlethal)</td>
</tr>
</tbody>
</table>

| Taxon                        | Hemolysisa      | Hemagglutination b | Suscep-
tibility to 500 U of penicillin per mlb |
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<tbody>
<tr>
<td>F. pseudonecrophorum</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F. necrophorum biocar A</td>
<td>β</td>
<td>β</td>
<td>+</td>
</tr>
<tr>
<td>F. necrophorum biocar B</td>
<td>β</td>
<td>β</td>
<td>–</td>
</tr>
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a –, Nonhemolytic; β, β-hemolytic.
b Determined by the slide glass method.

TABLE 1. Biochemical and biological characteristics of F. pseudonecrophorum and F. necrophorum

TABLE 2. Levels of DNA-DNA homology between F. pseudonecrophorum and F. necrophorum strains

<table>
<thead>
<tr>
<th>Unlabeled DNA from:</th>
<th>G+C content (mol %)</th>
<th>% Homology with labeled DNA from:</th>
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<tr>
<td>Species</td>
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<td>32.0</td>
<td>13.8</td>
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TABLE 3. Differential characteristics of F. pseudonecrophorum and F. necrophorum

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<thead>
<tr>
<th>Taxon</th>
<th>Hemolysisa</th>
<th>Hemagglutination b</th>
<th>Susceptibility to 500 U of penicillin per mlb</th>
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<td>β</td>
<td>β</td>
<td>+</td>
</tr>
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<td>β</td>
<td>β</td>
<td>–</td>
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</tbody>
</table>
homology values may have to be assessed differently for each bacterial group.

In this study, the strains of biovars A and B exhibited more than 70% DNA homology to each other; however, the three biovar C strains exhibited only low levels of DNA homology to the strains of the other two biovars, whereas they exhibited high levels of DNA homology to reference strain Fn521\(^\text{T}\). Biovar C is biochemically similar to the other two biovars but is distinct in genetic terms.

It is quite possible that the three biovars do not constitute a single DNA relatedness group. We suggest that nonhemolytic biovar C organisms belonging to \textit{F. necrophorum} should be established as an independent new species, \textit{Fusobacterium pseudonecrophorum}, named for "\textit{Actinomyces pseudo-necro-phorus}," which was first isolated and named by Harris and Brown in 1927 (10).

**Description of \textit{Fusobacterium pseudonecrophorum} sp. nov., nom. rev. (ex \textit{PrCvot 1940}). \textit{Fusobacterium pseudonecrophorum} (pse.do.ne.cro'pho.rum. Gr. adj. \textit{pseudes}, false; Gr. adj. \textit{necros}, dead; Gr. adj. \textit{phorum}, bearing; M.L. neut. adj. \textit{pseudonecrophorum}, false necrosis producing) is an obligatory anaerobic, gram-negative, nonsporulating, rod-shaped organism; the cells are thick, semilong rods and are filamentous. After 3 days of incubation, colonies on GAM agar supplemented with 5% defibrinated horse blood are about 2.5 mm in diameter, umbonate, grayish, and translucent. The margins are undulate, and the surfaces are granular. This species exhibits no hemolysis with the blood of rabbits and horses. All of the strains produce butyric acid from glucose and propionic acid from threonine and lactic acid. They are negative for esculin hydrolysis and positive for indole production. The results of tests for starch hydrolysis, motility, nitrate reduction, gas formation, catalase production, \(H_2\)S production, and growth in 20% bile are consistent with the description of \textit{F. necrophorum} in \textit{Berger's Manual of Systematic Bacteriology} (15). They are negative in the lipase reaction. Each strain produces acid from glucose and fructose (weak). Acid is not produced from amygdalin, lipase reaction. Each strain produces acid from glucose and with the description of \textit{F. necrophorum}.

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The \(G+C\) content is 28.0 to 30.4 mol%.

The type strain of \textit{F. pseudonecrophorum} sp. nov., nom. rev. (ex \textit{PrCvot 1940}) is strain Fn521 (= JCM 3722).

**Acknowledgment**

We are grateful to R. Harasawa, Department of Veterinary Microbiology, Miyazaki University, for his advice on the DNA-DNA hybridization test.

**Literature Cited**