Fusobacterium pseudonecrophorum Is a Synonym for *Fusobacterium varium*

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DNA-DNA hybridization studies (with the S1 nuclease method [J. L. Johnson, *Methods Microbiol.* 18:33-74, 1985]) were performed on members of the genus *Fusobacterium*. *Fusobacterium varium* ATCC 8501^T showed 88 and 79% DNA homology with *F. pseudonecrophorum* JCM 3722^T and JCM 3723, respectively, while *F. pseudonecrophorum* JCM 3722^T showed 81 and 82% DNA homology with *F. varium* ATCC 8501^T and *F. pseudonecrophorum* JCM 3723, respectively. These genetic data and their similar phenotypic characteristics suggest that *F. varium* (Eggerth and Gagnon 1933) Moore and Holdeman 1969 and *F. pseudonecrophorum* (ex Prévot 1940) Shinjo et al. 1990 belong to a single species. We propose, therefore, that strains JCM 3722 and JCM 3723 of *F. pseudonecrophorum* be transferred to the species *F. varium*.

In 1990, Shinjo et al. (16) proposed that biovar C of *Fusobacterium necrophorum* (Flügge) Moore and Holdeman be recognized as *F. pseudonecrophorum* sp. nov., nom. rev. (ex Prévot 1940). In that communication, they compared *F. necrophorum* biovars A, B, and C and presented DNA-DNA hybridization data which separated nonhemolytic biovar C from the other, hemolytic biovars. They concluded that while biovar C was "biochemically similar to the other two biovars," it was distinct in genetic terms and was "worthy of species designation." This species was first described in 1927 from puerperal infection of women and named *Actinomyces pseudonecrophorus* (5). Subsequently, it was renamed *Sphaerophorus pseudonecrophorus* (13) and bovine and ovine strains originally identified as *S. necrophorus* were examined in the recent description of *F. pseudonecrophorum*. However, while members of the genus *Fusobacterium* exhibit substantial intrageneric heterogeneity on the basis of 16 rRNA sequences (9), the currently used phenotypic tests (12) fail to discriminate among *Fusobacterium* species. For example, cat strains of *F. alocis* cannot be distinguished from *F. simiae* or *F. necrophorum* on the basis of these phenotypic tests (11). Furthermore, many of the phenotypic tests considered discriminatory of species (12) depend for their accuracy on the use of identical substrates to ensure that any variations seen are not simply a reflection of different or preferential utilization of components in the basal medium used. Therefore, accurate identification of members of the genus *Fusobacterium* to the species level requires extensive genetic testing with care to include known type strains in each analysis. To identify the equine *Fusobacterium* strains isolated in previous studies (2) to the species level, we included phenotypic and genotypic analyses of the type strains of *F. pseudonecrophorum* (16). This report describes results of our DNA relatedness studies on strains of *F. pseudonecrophorum* JCM 3722^T and JCM 3723 and a large number of the described species of the genus. In addition, phenotypic tests of these strains were performed to check the discrepancies reported in the literature (15, 16).

MATERIALS AND METHODS

Bacterial strains. A total of 19 strains were included in this study. Detailed phenotypic characterization was performed on *F. pseudonecrophorum* Japanese Culture Collection of Microorganisms (JCM) 3722^T and JCM 3723, *F. necrophorum* subsp. *funduliforme* JCM 3724^T (obtained from JCM); *F. varium* Virginia Polytechnic Institute (VPI) 0501 (= ATCC 8501^T) (obtained from the late E. P. Cato), and *F. necrophorum* subsp. *necrophorum* ATCC 25286^T (obtained from ATCC). In addition, the following strains were included in the DNA hybridization study: *F. gonidiaformans* VPI 0482A (= ATCC 25563^T), *F. mortiferum* VPI 4123A-2 (= ATCC 25557^T), *F. necrogenes* VPI 2368 (= ATCC 25556^T), *F. perfoetens* VPI 11077 (= ATCC 29250^T), *F. periodonticum* VPI 13726 (= ATCC 33963^T), and *F. simiae* VPI 13611 (= ATCC 33568^T) (obtained from E. P. Cato). *F. naviforme* ATCC 25832^T, *F. russii* ATCC 25533^T, and *F. nucleatum* subsp. *nucleatum* ATCC 25586^T were obtained from ATCC. In addition, *F. alocis* Veterinary Pathology and Bacteriology Culture Collection (VPB) 3403 and *Fusobacterium* sp. strains VPB 3331, VPB 3379, VPB 3942, and VPB 3948, unidentified on the basis of DNA homology with *Fusobacterium* sp. type strains (11), were included.

Culture media and methods. The general methods used for growth and biochemical characterization have been described previously (11), except that metabolic fatty acids were detected by gas-liquid chromatography on a Hewlett-Packard model 5890 series II gas chromatograph incorporating a flame ionization detector and fitted with a Hewlett-Packard 7673 Automatic Sampler. Samples were chromatographed on a 2-m column, internal diameter 2 mm, packed with 10% AT 1200 plus 1% H₃PO₄ on Chromosorb WAW 80/100 mesh (Alltech).

Preparation and analysis of cell wall fatty acids. Cells were harvested from sheep blood agar plates (1) and processed as described previously (10). In addition, the following fatty acid standards, obtained from Larodan, were included: 10-methyldodecanoate (catalog no. 21:1210), 12-methyldodecanoate (catalog no. 21:1211), 14-methylhexadecanoate (catalog no. 21:1614), and 3-hydroxyhexadecanoate (catalog no. 24:1603).

Alloenzyme electrophoresis. Cells were inoculated into prerduced brain heart infusion broth, harvested following 6 h of growth at 37°C, and processed with glutamate dehydro-

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TABLE 1. Metabolic fatty acids, selected biochemical tests, and penicillin susceptibility of selected *Fusobacterium* species^a

Bacterial strain	Propionic acid (µg/ml) from:		Acid from glucose ^b	Lipase
	Lactate	Threonine		
<i>F. varium</i> ATCC 8501 ^T	0	21.8	—	—
<i>F. pseudonecrophorum</i> JCM 3722 ^{Tc}	0	3.6	W	—
<i>F. pseudonecrophorum</i> JCM 3723	0	6.6	W	—
<i>F. necrophorum</i> subsp. <i>necrophorum</i> ATCC 25286 ^{Td}	63.4	17.5	—	+
<i>F. necrophorum</i> subsp. <i>funduliforme</i> JCM 3724 ^{Te}	56.7	7.4	—	+

^a Growth of all strains was unaffected by bile. When grown in cooked meat glucose, all strains produced major amounts of acetic, butyric, and, with the exception of *F. varium* ATCC 8501^T (which produced a minor amount), propionic acids. All strains produced indole and gas in peptone yeast glucose deeps. All strains were susceptible to penicillin (2 U/ml). No strain hydrolyzed esculin or starch, reduced nitrate to nitrite, or produced catalase or acid from amygdalin, arabinose, cellobiose, esculin, fructose, lactose, mannose, melibiose, raffinose, salicin, starch, sucrose, or xylose.

^b —, pH >5.7; W, pH >5.5 but <5.7;

^c *F. necrophorum* biovar C;

^d *F. necrophorum* biovar A;

^e *F. necrophorum* biovar B (15, 16).

genase as described previously (10). In addition, 2-oxoglutarate reductase was detected as described by Gharbia and Shah (4).

DNA hybridization. The method used for DNA isolation was essentially as described previously (11), except that the hydroxyapatite procedure (8) was used to isolate DNA from JCM 3722^T and JCM 3723. All DNA preparations were sheared by being passed three times each through a French pressure cell at 16,000 lb/in² and were denatured by being heated at 100°C for 5 min. Small amounts (5 µg) of the fragmented and denatured DNA preparations were labeled with ¹²⁵I basically as described previously (14). Each of the reassociation vials contained 10 µl (10 ng; 30,000 cpm) of labeled DNA, 50 µl (20 µg) of unlabeled DNA, 50 µl of 5.28 M NaCl containing 5 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer (pH 7.0), and 25 µl of deionized formamide. These vials were incubated at 50°C for 20 h. The S1 nuclease procedure was used for DNA similarity determinations (8). Labeled reference DNA preparations reassociated in the presence of 20 µl of salmon sperm DNA resulted in background values which were always less than 10% of the values obtained when the preparations were reassociated in the presence of unlabeled reference DNA. The results shown (see Table 2) are means of two determinations in which DNA from each of the strains labeled was extracted from two different batches of cells on separate occasions and independently iodinated on two occasions.

RESULTS AND DISCUSSION

The cellular and colonial morphologies of *F. varium* and *F. pseudonecrophorum* were as previously reported (12, 16). The results of our metabolic fatty acid, biochemical, and penicillin sensitivity studies on strains previously identified as *F. necrophorum* biovars A, B, and C and *F. varium* are given in Table 1. In addition, the cellular fatty acid profiles of these three isolates were similar to each other and to those

TABLE 2. Levels of DNA homology of *F. varium* and *F. pseudonecrophorum* with other *Fusobacterium* sp. reference and type strains.

Source of unlabelled DNA	% Homology with labeled DNA ^a from:	
	<i>F. varium</i> ATCC 8501 ^T	<i>F. pseudonecrophorum</i> JCM 3722 ^T
<i>F. varium</i> ATCC 8501 ^T	100	81
<i>F. pseudonecrophorum</i> JCM 3722 ^T	88	100
<i>F. pseudonecrophorum</i> JCM 3723	79	82
<i>F. necrophorum</i> subsp. <i>necrophorum</i> ATCC 25286 ^T	6	1
<i>F. necrophorum</i> subsp. <i>funduliforme</i> JCM 3724 ^T	5	1
<i>F. alocis</i> VPB 3403	8	5
<i>F. simiae</i> ATCC 33568 ^T	6	5
<i>F. necrogenes</i> ATCC 25556 ^T	9	8
<i>F. perfoetens</i> ATCC 2925 ^T	7	4
<i>F. periodontium</i> ATCC 33963 ^T	27	36
<i>F. gonadiformans</i> ATCC 25563 ^T	7	3
<i>F. russii</i> ATCC 2553 ^T	5	4
<i>F. nucleatum</i> subsp. <i>nucleatum</i> ATCC 25586 ^T	5	5
<i>F. mortiferum</i> ATCC 25557 ^T	25	20
<i>F. naviforme</i> ATCC 25832 ^T	2	<1
<i>Fusobacterium</i> sp. strains		
VPB 3331	<1	<1
VPB 3379	4	2
VPB 3942	3	4
VPB 3948	5	3

^a The percent reassociation values for reference DNA preparations were normalized to 100% homology.

reported previously for *F. varium* (3, 6, 17). These phenotypic results showed that strains previously described as *F. pseudonecrophorum* were similar to *F. varium*. However, in our study *F. varium* ATCC 8501^T and *F. pseudonecrophorum* JCM 3722^T and JCM 3723 gave results similar to one another in phenotypic tests and substantially similar results to those described previously for *F. varium* (12), while *F. pseudonecrophorum* JCM 3722^T and JCM 3723 gave results for lactate conversion to propionate, susceptibility to penicillin, growth in bile, and glucose and fructose fermentation at variance with those reported in describing the species (16). Furthermore, our studies confirmed that *F. necrophorum* subsp. *funduliforme* JCM 3724^T was lipase positive, as reported by Shinjo et al. (16), but at variance with a subsequent report (15) in which it was reported to be lipase negative. Given the similar phenotypic characteristics of *F. varium* and *F. pseudonecrophorum*, an additional phenotypic comparison was performed. Alloenzyme electrophoresis of ATCC 8501^T, JCM 3722^T, and JCM 3723 detected glutamate dehydrogenase and 2-oxoglutarate reductase in each as a single electrophoretic type with the same mobility. When DNA-DNA hybridization was performed, our studies showed that labeled DNA from *F. varium* ATCC 8501^T had 88 and 79% homology with unlabeled DNA from *F. pseudonecrophorum* JCM 3722^T and JCM 3723, respectively (Table 2). Similarly, labeled DNA from *F. pseudonecrophorum* JCM 3722^T showed 81 and 82% homology with unlabeled DNAs from *F. varium* ATCC 8501^T and JCM 3723, respectively. It is considered that strains of bacteria which demonstrate DNA homology equal to or greater than 60% by the methods used in our studies belong to the same species (7). On the basis of the phenotypic similarities and DNA homol-

ogy, it is our contention that JCM 3722^T and JCM 3723 are strains of *F. varium* and do not belong to a separate species. These results question the validity of the species *F. pseudonecrophorum* (16) or, at least, the inclusion of JCM 3722 as the type strain of the species and the inclusion of JCM 3723 in that species. The possibility exists, however, that the strains originally described in 1927 as *A. pseudonecrophorus* (and not included in either the report of Shinjo et al. [16] or this report) are different and a distinct species.

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