Fusobacterium nucleatum subsp. fusiforme subsp. nov. and Fusobacterium nucleatum subsp. animalis subsp. nov. as Additional Subspecies within Fusobacterium nucleatum

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Using a variety of physiological, biochemical, and molecular systematic analyses, we have shown previously that there are four groups within the species Fusobacterium nucleatum. Two of these groups correspond to the recently proposed taxa F. nucleatum subsp. nucleatum and F. nucleatum subsp. polymorphum. In this paper we show that the two remaining groups are distinct and formally propose that they should be recognized as F. nucleatum subsp. fusiforme (type strain, NCTC 11326) and F. nucleatum subsp. animalis (type strain, NCTC 12276). The tests which we used did not allow a full assessment of the status of F. nucleatum subsp. vincentii compared with F. nucleatum subsp. nucleatum.

Fusobacterium nucleatum, the type species of the genus Fusobacterium, has been shown to comprise a heterogeneous collection of strains (1, 3, 4). Recently, Dzink et al. (2) described three subspecies, F. nucleatum subsp. nucleatum, F. nucleatum subsp. polymorphum, F. nucleatum subsp. vincentii, while almost simultaneously we described four subspecies (5) following a series of detailed systematic analyses (3–6, 8). The descriptions of F. nucleatum subsp. nucleatum and F. nucleatum subsp. polymorphum in two previous studies (2, 5) appear to be consistent, and the same type strains and the same names were proposed independently for each subspecies. It is evident from a previous description of “F. nucleatum subsp. animalis” (5) that this organism is a distinct taxon within the species F. nucleatum. However, the taxonomic position of “F. nucleatum subsp. fusiforme” (5) remains unclear. Because of the paucity of tests used for describing F. nucleatum subsp. vincentii, it is uncertain whether this subspecies and F. nucleatum subsp. fusiforme are synonymous. In this study, we compared strains of F. nucleatum subsp. fusiforme with the type strain of F. nucleatum subsp. vincentii by using enzymic electrophoretic patterns, which have been shown previously to have significant intraspecies diagnostic value in the genus Fusobacterium. In addition, in this paper we formally propose recognition of F. nucleatum subsp. animalis. Our proposals are consistent with our recent rRNA sequence data (9).

MATERIALS AND METHODS

Bacterial strains. F. nucleatum reference strains ATCC 25586T (T = type strain), ATCC 49256T, ATCC 10953T, NCTC 11326T, and NCTC 12276T and 10 clinical isolates resembling strains NCTC 11326T and NCTC 12276T were used in this study.

Growth conditions and identification. All strains were maintained by weekly subculturing on blood agar culture medium containing 5% (vol/vol) sheep blood (Oxoid) in an atmosphere containing 10% CO₂ and 10% H₂ in N₂ at 37°C. Clinical isolates were identified as described previously (4, 7).

Electrophoresis and staining of enzymes. Cell extracts were prepared and electrophoresis was carried out on cellophane support strips as described previously (3). Glutamate dehydrogenase (GDH) and 2-oxoglutarate reductase (OGR) bands were stained as described previously (3, 4).

RESULTS AND DISCUSSION

All of the isolates were identified as members of F. nucleatum by their ability to produce indole, their absence of urease activity, and their ability to reduce nitrate. Butyrate and acetate were major acidic end products of metabolism. Six of the clinical isolates which we tested morphologically resembled strain NCTC 11326T (previously designated F. nucleatum subsp. fusiforme); the electrophoretic mobilities for GDH and OGR for these strains were 1.8 and 2.0 cm, respectively. The isolates that resembled strain NCTC 12276T (designated F. nucleatum subsp. animalis) grew in the presence of 20% bile and had both GDH and OGR activities (electrophoretic mobilities, 3.2 and 3.8 cm, respectively). Figure 1 shows the GDH electrophoretic mobilities for the five reference strains representing the subspecies examined in this study. F. nucleatum subsp. polymorphum strains had a distinct electrophoretic mobility, whereas the GDH of F. nucleatum subsp. vincentii coelectrophoresed with F. nucleatum subsp. nucleatum GDH. The OGR of F. nucleatum subsp. nucleatum and the OGR of the type strain of F. nucleatum subsp. vincentii also comigrated (Fig. 1).

In this study we could not distinguish between strains of F. nucleatum subsp. nucleatum and the type strain of F. nucleatum subsp. vincentii. This could indicate that these two subspecies are synonymous, that an incorrect reference strain of F. nucleatum subsp. vincentii has been deposited in a culture collection, or that the tests which we used cannot be used to differentiate these taxa. However, it is clear that F. nucleatum subsp. vincentii and F. nucleatum subsp. fusiforme are not synonymous as is sometimes stated (12).

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The strains assigned to *F. nucleatum* subsp. *animalis* had the fastest-migrating enzymes on electropherograms and formed a distinct cluster that was readily recognizable from the other subspecies. When both GDH and OGR electrophoretic patterns were used as a basis for comparison, the strains assigned to *F. nucleatum* subsp. *fusiforme* (including strain NCTC 11326) were clearly different from *F. nucleatum* subsp. *vincentii* ATCC 49256; thus, separate subspecies status for *F. nucleatum* subsp. *fusiforme* was justified.

In previous studies we reported the absence of any clear physiological tests that can be used to differentiate among the subspecies of *F. nucleatum*. Furthermore, the levels of DNA-DNA homology when experiments are carried out under optimal hybridization conditions are high (4). Under stringent hybridization conditions, however, four centers of variations are clearly evident; these correlate with the groups formed on the basis of the GDH and OGR electrophoretic patterns (4). These groups have also been substantiated by rRNA gene restriction patterns, using a plasmid (pK3535) to probe chromosomal DNA digests of the subspecies (8). More recently, we found high levels of rRNA sequence similarity (ca. 97.3 to 98.4%) that are consistent with a single species. However, an unrooted phylogenetic tree based on *K* _nuc_ (envolutionary distance) values clearly delineated the four taxa. Therefore, we formally propose that *F. nucleatum* subsp. *fusiforme* and *F. nucleatum* subsp. *animalis*, which until now have been unclassified, should be recognized as new subspecies of *F. nucleatum*. Descriptions of these two taxa are given below.

Description of *Fusobacterium nucleatum* subsp. *fusiforme* subsp. nov., nom. rev., comb. nov. *Fusobacterium nuclea-
tum* subsp. *fusiforme* (fu.si.for’m.e. L.n. fusus, a spindle; L.n. forma, shape; M.L. adj. fusiforme, spindle shaped). Gram negative. Anaerobic. Cells are rod shaped and nonmotile, do not form filaments, and are 8 to 16 μm long by 0.5 μm in diameter. Colonies on blood agar after 24 h are granular with very irregular edges, 10 to 13 μm in diameter, and low cone shaped, and pitting is frequently observed if colonies are dislodged. Tryptase, protease peptone, and Bacto Casitone (Difco) markedly enhance growth in liquid medium. Few amino acids (mainly glutamate, histidine, cysteine, and serine) are utilized. The terminal pH in a glucose medium is higher than 7.0. The electrophoretic mobilities of GDH and OGR are 1.8 and 2 cm, respectively. Moderate levels of H₂S are produced in liquid medium. All strains possess the constitutive enzymes leucine aminopeptidase and gamma-glutamyl aminopeptidase. Serine-valine aminopeptidase and proline aminopeptidase are not produced. Glycosidases are absent.

The cell wall peptidoglycan contains meso-lanthionine as its diamino acid. Possesses straight-chain, monounsaturated, and hydroxylated long-chain fatty acids but not methyl-branched acids. Tetradecenoic acid (C₁₄:0; ca. 24 to 30%) and hexadecanoic acid (C₁₆:0; 20 to 26%) are the predominant nonhydroxylated acids. The major hydroxylated fatty acids are 3-hydroxytetradecanoic acid (3OH-C₁₄:0; ca. 12 to 18%) and 3-hydroxyhexadecanoic acid (3OH-C₁₆:0; 4.0 to 7%). The DNA base composition is 26 to 28 mol% G+C, as determined by the thermal denaturation method. The type strain is strain NCTC 11326.

Description of *Fusobacterium nucleatum* subsp. *animalis* subsp. nov. *Fusobacterium nucleatum* subsp. *animalis* (a.ni.ma’lis. L. n. animal, animal; *animalis*, of an animal). Gram negative. Anaerobic. Cells are nonmotile rods that occasionally form filaments which are up to 25 μm long. Colonies on blood agar are round, smooth, glistening, convex, translucent white, and 1.0 to 2.0 μm in diameter. Growth is markedly affected by the addition of Tryptase, protease peptone, and Bacto Casitone (Difco). Few amino acids (mainly histidine, arginine, cysteine, and, to a lesser extent, glutamate) are utilized. The terminal pH in a glucose liquid medium ranges between 6.8 and 7.0. Both GDH and OGR are present and exhibit fast electrophoretic mobilities (3.2 and 3.8 cm, respectively). Low levels of H₂S are produced, and growth is not affected by the addition of 20% bile. Strains are morphologically similar to *F. nucleatum* subsp. *polymorphum*, but the level of DNA-DNA homology between these two subspecies ranges between 60 and 67%. All strains possess the constitutive enzymes leucine aminopeptidase and gamma-glutamyl aminopeptidase. Serine-valine aminopeptidase and proline aminopeptidase are not produced. Glycosidases are absent.

The cell wall peptidoglycan contains meso-lanthionine as its diamino acid. Possesses straight-chain monounsaturated, and hydroxylated long-chain fatty acids but not methyl-branched acids. Tetradecenoic acid (C₁₄:0; ca. 24 to 30%) and hexadecanoic acid (C₁₆:0; 20 to 26%) are the predominant nonhydroxylated acids. The major hydroxylated fatty acids are 3-hydroxytetradecanoic acid (3OH-C₁₄:0; ca. 12 to 18%) and 3-hydroxyhexadecanoic acid (3OH-C₁₆:0; 4.0 to 7%). The DNA base composition is 26 to 28 mol% G+C, as determined by the thermal denaturation method. The type strain is strain NCTC 12276.

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REFERENCES


