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 of strains 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 cellulose 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 coelectrophoresed (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).
NEW FUSOBACTERIUM NUCLEATUM SUBSPECIES

Descriptions of these two taxa are given below.

Description of Fusobacterium nucleatum subsp. fusiforme subsp. nov., nom. rev., comb. nov. Fusobacterium nucleat-
REFERENCES


