NOTE***Eubacterium minutum* is an earlier synonym of *Eubacterium tardum* and has priority**

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The recently proposed species *Eubacterium minutum* and *Eubacterium tardum* appeared to be similar from their published descriptions. The aim of this study was to perform phenotypic and genetic analyses of strains of both species to clarify their taxonomic position. The type strains of *E. minutum* and *E. tardum* exhibited identical biochemical and protein profiles and their 16S rRNA gene sequences displayed 99.9% similarity. The G+C content of the DNA of both strains was estimated at 45 mol%. It is concluded that *E. minutum* and *E. tardum* are synonyms; *E. minutum* has priority. An emended description of *E. minutum* is given.

Keywords: *Eubacterium*, 16S rRNA, taxonomy

The genus *Eubacterium* is extremely heterogeneous, primarily because it has been a repository for organisms not corresponding to other, better-defined genera (Noble & Wade, 1998). Many of the asaccharolytic species are significantly associated with periodontitis, endodontic infections and oral abscesses but are rarely found in oral health (Wade, 1997). Strains tend to be slow-growing and biochemically unreactive and, because of this, there are a large number of unnamed taxa. Recently, a number of species have been described including *Eubacterium exiguum* (Poco *et al.*, 1996b), *Eubacterium minutum* (Poco *et al.*, 1996a), *Eubacterium infirmum* and *Eubacterium tardum* (Cheeseman *et al.*, 1996). From their published descriptions, *E. minutum* and *E. tardum* appear to be phenotypically similar, prompting the suspicion that they are one species. The aim of this study was to compare the type (and other) strains of *E. minutum* and *E. tardum* by a variety of phenotypic and genetic methods.

Bacterial strains

E. minutum ATCC 700079^T and ATCC 700080 and *E. tardum* NCTC 12941^T and SC88P were included in the study. Strains were maintained on Fastidious Anaerobe agar plus 5% horse blood under anaerobic conditions.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene of *E. minutum* ATCC 700079^T is AJ005636.

Metabolic end-product analysis

Bacterial strains were grown in peptone/yeast extract/glucose (PYG) broth and short-chain volatile and non-volatile fatty acids were extracted by standard methods (Holdeman *et al.*, 1977). Analysis was performed by gas chromatography with a capillary column packed with a 10% free-fatty-acid solid phase.

Protein and enzyme profiles

Profiles of whole-cell proteins were generated by SDS-PAGE using 10–15% gradient gels and the Phast-System (Pharmacia) as described previously (Slayne *et al.*, 1990) except that the cells were pre-treated with lysozyme (50 µg ml⁻¹) for 3 h at 37 °C.

Enzyme profiles were generated with the Rapid ID 32A anaerobe identification kit (bioMérieux) according to the manufacturer's instructions. Bacteria were harvested from blood agar plates [blood agar base no. 2 (LabM) plus 5% horse blood] incubated anaerobically at 37 °C for 72 h.

16S rRNA gene PCR and sequence analysis

16S rRNA genes were amplified by PCR using primers 27F and 1525R (Lane, 1991). PCR amplification was performed with an Uno II Thermocycler (Biometra) using PCR buffer (Bioline) containing 1.5 mM MgCl₂, 200 µM dNTPs, 1 mM of each oligonucleotide primer, 1 U *Taq* DNA polymerase (Bioline) and template

Fig. 1. SDS-PAGE protein profiles of *E. minutum* and *E. tardum*. Lanes: m, molecular mass markers; a, *E. tardum* SC88P; b, *E. tardum* NCTC 12941^T; c, *E. minutum* ATCC 700079^T; d, *E. minutum* ATCC 700080.

DNA in a total volume of 100 µl. Thirty amplification cycles were performed with a denaturing temperature of 94 °C for 1 min, annealing at 54 °C for 1 min and elongation at 72 °C for 2 min. PCR products were sequenced directly using a dye terminator cycle sequencing kit with AmpliTaq FS (Perkin Elmer) and 3 µl of a 20 ng template ml⁻¹ solution, according to the manufacturer's instructions. Sequencing was performed using an automated sequencer (ABI 377; Perkin Elmer) with primers 27F, 342R, 357F, 519R, 907R, 926F, 1100R, 1114F and 1392R (Lane, 1991).

Sequences were connected using DNASIS (Hitachi) and aligned by means of CLUSTAL W (Thompson *et al.*, 1994). The 16S rRNA gene of *E. tardum* NCTC 12941^T was resequenced in this study directly from PCR products; the gene had previously been sequenced from a clone. The nucleotide accession record, U13037, was updated.

Estimation of G + C content of DNA and DNA–DNA homology

G + C content of DNA was estimated by an HPLC method as described previously (Wade *et al.*, 1999). G + C estimations were repeated on three occasions. A spectrophotometric method (Huss *et al.*, 1983) was used to determine the DNA–DNA homology between the two species.

Synonymy of *E. minutum* and *E. tardum*

The Rapid ID 32A profiles for all four strains were 0000 0120 01. Poco *et al.* (1996a) reported the profile of *E. minutum* in this test to be 0000 0526 01, but incubated the test anaerobically. In this study, the test kit was incubated under aerobic conditions as recommended by the manufacturer. The protein profiles of the strains are shown in Fig. 1 and can be seen to be identical.

16S rRNA gene sequences of the two type strains differed by only two of 1437 unambiguously aligned bases (99.9% similarity) and the homology between the DNA preparations of the two strains was found to be 89%. These figures are both well within the range expected for strain variation within a species.

The G + C content of DNA was found to be 45 mol% for both *E. minutum* ATCC 700079^T and *E. tardum* NCTC 12941^T. Poco *et al.* (1996a) reported the G + C content of DNA of *E. minutum* strains as 38–40 mol%, calculated by the HPLC method employed here, while Cheeseman *et al.* (1996) reported a value of 45 mol% for *E. tardum* using the thermal denaturation method. In this study, the HPLC method was used and a figure of 45 mol% for both species was confirmed.

This study has shown that the type strains of *E. minutum* and *E. tardum* are identical and, by prior publication, *E. minutum* has precedence.

Emended description of *Eubacterium minutum*

The characteristics of the species are as described by Poco *et al.* (1996a) except that the G + C content of the DNA is 45 mol%.

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