NOTE *Bacteriology*

Comparison of the 16S-23S rRNA Intergenic Spacer Regions between *Fusobacterium varium* and "*Fusobacterium pseudonecrophorum*" Strains

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ABSTRACT. The 16S-23S rRNA intergenic spacer regions (ISRs) of five strains of "*Fusobacterium pseudonecrophorum*" which had been proposed as a new species, were compared with those of *F. varium* ATCC 8501^T. All the strains of "*F. pseudonecrophorum*" exhibited of sequence similarities of 97.7% to 100% to the strain of *F. varium* in their 16S-23S rRNA ISR sequences. This indicates that the strains of "*F. pseudonecrophorum*" and the type strain of *F. varium* are identical at the species level.

KEY WORDS: *Fusobacterium varium*, ISR, 16S-23S rRNA.

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We proposed "*Fusobacterium pseudonecrophorum*" as a new species for the biovar C of *F. necrophorum* based on the DNA-DNA homology analysis [12]. Recently, Bailey and Love [3] reported that "*F. pseudonecrophorum*" and *F. varium* were specifically related to each other from the results of DNA-DNA hybridization analysis. In their report, they proposed that "*F. pseudonecrophorum*" was a synonym of *F. varium*. However, the isolation sources and pathogenicity of the two species differed from each other [11]. The former was isolated from the lesions of various human organs and was experimentally pathogenic to the guinea pig and rabbit, while the latter was less pathogenic to these animals.

The 16S rDNA sequence is now well known and is applied in both phylogeny and the identification of prokaryotes. It is used for analysis at the genus level, and its intrachromosomal differences are small and can be dismissed in phylogenetic analysis. In addition, DNA analysis of the 16S-23S rRNA intergenic spacer region (ISR) was shown to be very useful in typing and identification of bacteria [5]. The ISR analysis is a more useful tool for closely related organisms such as *Listeria* and *Staphylococcus* than the 16S rRNA analysis because of its precise differentiation ability [5–7]. To date, limited data are available concerning the sequence analysis for fusobacterial species. Therefore, we evaluated the propriety of the new classification using the nucleotide sequences of the 16S-23S rDNA ISR among *F. varium* ATCC 8501^T and five "*F. pseudonecrophorum*" strains.

Six strains including the type strain ATCC 8501^T of *F. varium* and strains JCM3721, JCM3722, JCM3723, Fn525 and Fn526 of "*F. pseudonecrophorum*" were used in this study. All of these strains were inoculated to GAM agar (Nissui Seiyaku Co., Tokyo) supplemented with 5% horse blood and incubated anaerobically by the steel wool method at 37°C for 72 hr [1]. The developed colonies were subcultured in BPPY medium [10] at 37°C for 24 hr, and the cultures were used for this study. Bacterial DNA was isolated and purified by phenol/chloroform extraction and ethanol precipitation [2].

PCR amplification of the 16S-23S rRNA ISR was performed with the following primers: the forward primer Ec16S, 1390p, 5'-TTG TAC ACA CCG CCC GTC-3' [4]; and the reverse primer Ec23S, 441p, 5'-TAC TGG TTC ATA ATC GGT CA-3' [9]; having been described elsewhere. The PCR products were purified with a QIA quick PCR Purification Kit (QIAGEN) according to the manufacturer's instructions. The nucleotide sequences of the purified PCR products were determined by the direct sequencing method and aligned by using the CLUSTAL W program [13].

Two bands of PCR products (long and short) were observed for all the strains examined. The amplified products approximated to 700 bp and 800 bp in length. The short bands (ca. 700 bp) showed higher intensities than the long ones (ca. 800 bp). Only the short bands of the two PCR product sequences were used for the sequence analysis. According to the rDNA sequence of *F. varium*, available in the DDBJ/EMBLE/GENBANK (accession number AF342853), the 5' and 3' termini of the 16S-23S rRNA ISRs were specifically located as shown in Fig. 1. The lengths of the ISR from the shorter PCR products were subsequently found to be 171 bp for *F. varium* ATCC 8501^T and 173 bp for the five strains of "*F. pseudonecrophorum*"·*F. varium* and "*F. pseudonecrophorum*" strains exhibited similarities of 97.7% to 100%, based on the alignment shown in Fig. 1. For estimating the copy number of the 16S rDNA in all strains, the genomic DNAs were digested with the restriction enzyme *Hin*dIII and then applied to Southern hybridization with PCR-amplified 16S rDNA of *F. varium* ATCC 8501^T as a probe. All strains were shown to have two

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                3' end of 16S rRNA gene
                ------>
ATCC8501T: ACCTCCTTTCTAAG-AG-TCATTTCTCTTTCTCTATTCTATTGATAATGTTCTTGCAGCGTATTAGC-GCTGGATAACTTATCAAA
JCM3721  : .............G..A.....................................................-.................
JCM3722  : .............G..A.....................................................-.................
JCM3723  : ... .........G..A.....................................................-.................
Fn525    : .............G..A.....................................................C.................
Fn526    : .............G..A.....................................................C.................

ATCC8501T: TGGACATTGGAAACTATATAGTAGATAATACAATTTTAACTCTTGTTTATATAAACAGAGTTAGCTGTCAATCAAATTAAACAAAA
JCM3721  : ................................C.....................................................
JCM3722  : .....................................................................................
JCM3723  : .....................................................................................
Fn525    : ..................................................................................-.....
Fn526    : ..................................................................................-.....
                5 'end 23S rRNA gene
                <------
ATCC8501T: ACAAAATAGGTTAAA : 171
JCM3721  : ............... : 173
JCM3722  : ............... : 173
JCM3723  : ............... : 173
Fn525    : ............... : 173
Fn526    : ............... : 173
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Fig. 1. Alignment of 16S-23S ISR sequences of *F. varium* ATCC 8501^T, and "*F. pseudonecrophorum*" strains, JCM3721, JCM3722, JCM3723, Fn525, and Fn526. The length of the ISR is noted at the end of the sequences. The complete ISR sequence between the end of the 16S rRNA gene and the beginning of the 23S rRNA gene is shown. Nucleotides, identical to *F. varium* ATCC 8501^T are marked by dots. Hyphens represent alignment gaps.

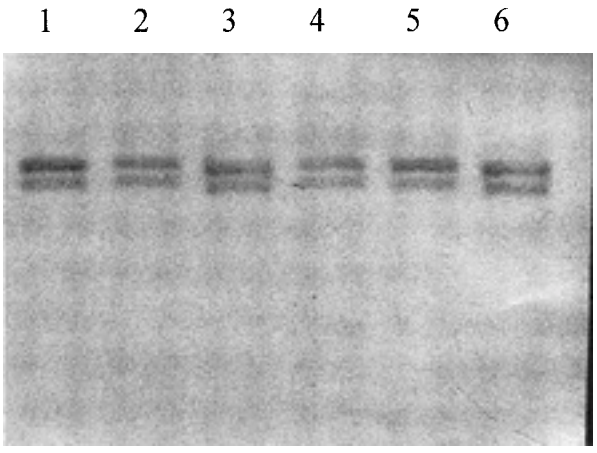

Fig. 2. Southern hybridization of *F. varium* and "*F. pseudonecrophorm*" with the PCR-amplified 16S rDNA of *F. varium* ATCC 8501^T as a probe. The genomic DNA was digested with *Hin*dIII. 1. *F. varium* ATCC 8501^T, "*F. pseudonecrophorum*" strains JCM3722 (2), JCM3723 (3), JCM3721 (4), Fn525 (5), and Fn526 (6).

copies of rDNA alleles on their genomes (Fig. 2). However, there was no tRNA-like structure within the ISR sequences as shown in Fig. 1 when the tRNAscan-SE program [8] was used.

In conclusion, no significant difference at the species level was observed between *F. varium* and "*F. pseudonecrophorum*" on the basis of sequence homologies in the 16S-23S rRNA ISR.

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