CONFIRMED NUCLEOTID SEQUENCE OF fanF OF ESCHERICHIA COLI K99 FIMBRIAE

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(Accepted for publication: Sept. 31, 1993)

Key words: Enterotoxigenic Escherichia coli, K99 fimbriae, fanF DNA sequence

Enterotoxigenic Escherichia coli possessing K99 fimbriae cause diarrhea in newborn calves, piglets and lambs⁹. These fimbriae have been found to bind specifically to N-glycolylyneuraminic acid-containing Gm₃ ganglioside⁴ and to consist of eight different subunits named FanA to FanH³. The nucleotide sequences and functions of these subunits were reported as follows: FanC is a major subunit called fimbrillin which forms the fimbrial structure⁴, FanA and FanB are regulatory proteins which control expression of fimbriae⁵,⁸, FanD is platform protein⁷, and FanG and FanH are minor subunits⁶. However, there is no available information about adhesin, which recognizes the host receptor ganglioside.

Recently, the nucleotide sequences of fanF, the gene encoding FanF, were reported by two different laboratories²,¹⁰, but a great difference was observed between nucleotides 769 and the 3′ end of the sequences of fanF described by the two laboratories. Thus the resultant size of the open reading frame representing FanF was reported to be 999 bp by Simons et al.¹⁰ and 813 bp by Ono et al.². Therefore, we confirmed the nucleotide sequence of this region.

pFK99 (pBR322), which contains the entire K99 fimbrial gene cluster (fanA to fanH)¹¹ was kindly supplied by Dr. F. K. de Graaf, Vrije University, Amsterdam, the Netherlands. The BamHI-BamHI fragment of pFK99 (pBR322), which contains the entire gene cluster, was cloned into pCU19 vector (pFK99 (pUC19)). Then the NheI-NspI fragment of pFK99 (pUC19), which contains the disputed region, was isolated by agarose gel electrophoresis and inserted into pUC18 cleaved with XbaI and SphI. DNA sequences were determined using a SEQUENASE Ver. 2.0 kit (TOYOBO Co. Ltd., Osaka, Japan).

The nucleotide sequence of fanF and the corresponding amino acid sequence are shown in Fig. 1. The upstream region from the NheI restriction site refers to that reported by Ono et al.². fanF encoded 333 amino acids. This confirmed nucleotide

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**fanE**

\[
\text{ACTAGTGGAAACATCACGGTAACAGCATTTAATGACTATGGCGTTGCAACCAGCTACACTGTTCAAAGGAGTAAATA}
\]

Thr Ser Gly Asn Ile Thr Val Thr Ala Phe Asn Asp Tyr Gln Val Ala Thr Ser Tyr Thr Val Gln Arg Ser Lys**

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**fanF**

\[
\text{ATG AAA AAT AAA TAT AAT TTA TTT TTT TTA CTT TCT TGT TGT TAT GGA GAT GTG GCG}
\]

Met Lys Asn Asp Asp Ala Ala Asp Gly Tyr Ala Gln Leu His

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**fanG**

\[
\text{ATA GAA ACA TTC CTG AAT GTC ACA ATG GAA CAT ATG TGATGAAAAAATTATATAAAGCAATA}
\]

Thr Val Ile Cys Asp Ala Asp Gln Ser Gly Trp Asp Gly Lys Arg Tyr Ala Gln Leu His

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**Fig. 1** Primary sequence of the **fanF** gene. The upstream region from the *NheI* restriction site refers to that reported by Ono *et al.* (1991). -35 and -10 indicate promoter sequences. S. D. means a ribosome-binding site.

The stopping codon is indicated by *****. The disputed region starts from nucleotide 769 (indicated by arrows).
sequence was the same as that reported by Simons et al. 10).

REFERENCES


