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## CONFIRMED NUCLEOTIDE SEQUENCE OF *fanF* OF *ESCHERICHIA COLI* K99 FIMBRIAE

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Enterotoxigenic *Escherichia coli* possessing K99 fimbriae cause diarrhea in newborn calves, piglets and lambs<sup>9)</sup>. These fimbriae have been found to bind specifically to N-glycolylneuraminic acid-containing GM<sub>3</sub> ganglioside<sup>1)</sup> and to consist of eight different subunits named FanA to FanH<sup>3)</sup>. The nucleotide sequences and functions of these subunits were reported as follows ; FanC is a major subunit called fimbillin which forms the fimbrial structure<sup>4)</sup>, FanA and FanB are regulatory proteins which control expression of fimbriae<sup>5,8)</sup>, FanD is platform protein<sup>7)</sup>, and FanG and FanH are minor subunits<sup>6)</sup>. 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 laboratories<sup>2,10)</sup>, 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.*<sup>10)</sup> and 813 bp by Ono *et al.*<sup>2)</sup>. Therefore, we confirmed the nucleotide sequence of this region.

pFK99 (pBR322), which contains the entire K99 fimbrial gene cluster (*fanA* to *fanH*)<sup>11)</sup> was kindly supplied by Dr. F. K. de Graaf, Vrije University, Amsterdam, the Netherlands. The *Bam*HI-*Bam*HI fragment of pFK99 (pBR322), which contains the entire gene cluster, was cloned into pCU19 vector (pFK99 (pUC19)). Then the *Nhe*I-*Nsp*7524I fragment of pFK99 (pUC19), which contains the disputed region, was isolated by agarose gel electrophoresis and inserted into pUC18 cleaved with *Xba*I and *Sph*I. 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 *Nhe*I restriction site refers to that reported by Ono *et al.*<sup>2)</sup>. *fanF* encoded 333 amino acids. This confirmed nucleotide

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*fanE* →                    -35                    -10                    S. D.

ACTAGTGAAACATCAGGTAACAGCATTAACTGACTATGGCGTTGCAACCAGCTACACTGTTCAAAGGAGTAAATA  
ThrSerGlyAsnIleThrValThrAlaPheAsnAspTyrGlyValAlaThrSerTyrThrValGlnArgSerLys\*\*

*fanF* →

1 ATG AAA AAT AAA TAT AAT TTA TTA TTT TTT TTA TTT CTT TTG TGT TAT GGA GAT GTG GCG  
\* Met Lys Asn Lys Tyr Asn Leu Ile Phe Phe Leu Phe Leu Leu Cys Tyr Gly Asp Val Ala  
61 CTG GCA GCA TGC ACA GGG AAA CTG AAA ATC TCA CCT GGT TAT AGT GGC CAT ACT TAT TCA  
Leu Ala Ala Cys Thr Gly Lys Leu Ile Ser Pro Gly Tyr Ser Gly His Thr Tyr Ser  
  
 121 TTT GAT TCC AGT ATT CCA AAT AAT AGT AAT ATA GCA AGA TAC CTG GTC GAA ATT TCT GAG  
Phe Asp Ser Ser Ile Pro Asn Asn Ser Asn Ile Ala Arg Tyr Leu Val Glu Ile Ser Glu  
  
 181 AAA ATT GTT TGT GAT GCG GAC CAG TCA GGC TGG GAT GGT AAA CGT TAT GCT CAA TTA CAT  
Lys Ile Val Cys Asp Ala Asp Gln Ser Gly Trp Asp Gly Lys Arg Tyr Ala Gln Leu His  
  
 241 CTT TAT TCA TCA GGT GCC TTA TGT GAA AGT GTC AGT GGA GAT GGG ATT ACA TTT AGG TCA  
Leu Tyr Ser Ser Gly Ala Leu Cys Glu Ser Val Ser Gly Asp Gly Ile Thr Phe Arg Ser  
  
 301 AAT GTG TCC GGG CTG TCA TGG CGT TTT CCC AAT GGC ATA CCA TAC CAC TGT GCA GCA GGC  
Asn Val Ser Gly Leu Ser Trp Arg Phe Pro Asn Gly Ile Pro Tyr His Cys Ala Ala Gly  
  
 361 CAA ATA AAT CTT GGC GGT ATA AAA TAT GCG GAT AGA AAT GGT AAA GTT ACC TGG AAT CCT  
Gln Ile Asn Leu Gly Ile Lys Tyr Ala Asp Arg Asn Gly Lys Val Thr Trp Asn Pro  
  
 421 GGT GAA CTA CGA CAT GAA ATA TTT TTA AGA GTG GAT AAC AGA TTT GAT TTC AGT AAA AGC  
Gly Glu Leu Arg His Glu Ile Phe Leu Arg Val Asp Asn Arg Phe Asp Ser Lys Ser  
  
 481 AGA ACA TTT TCT GTA AAC ACA ATT TCT GGT AGA GGA GGA TTA GGT GGA GAC AGC TCA GTA  
Arg Thr Phe Ser Val Asn Thr Ile Ser Gly Arg Gly Leu Gly Asp Ser Ser Val  
  
 541 GTT ATA CCT CTC ATA GGG AGT TCA TTT AAC TAT TCC TAT TCT AAC ATC GCT ACC TGC ACT  
Val Ile Pro Leu Ile Gly Ser Ser Phe Asn Tyr Ser Tyr Ser Asn Ile Ala Thr Cys Thr  
  
 601 TTG ACT GGC CCA AGT GAA GTG AAT TTC AAC ACT GTA ACC ACG TCA GAT GTA CTC AAA GGA  
Leu Thr Gly Pro Ser Glu Val Asn Phe Asn Thr Val Thr Ser Asp Val Leu Lys Gly  
  
 661 ACA ACA CAT CGT GAT CTT AAC TTA AGG GCA GAA TGT AGG AAC AGG GGG GCT AGC TTA GGA  
Thr Thr His Arg Asp Leu Asn Leu Arg Ala Glu Cys Arg Asn Arg Gly Ala Ser Leu Gly  
NheI  
Disputed region →  
 721 CTC AAT TTT AAA TTT GAG CCT CAG TAT AAA GAT GTT TCT GCA AAT AAA CAG GGC GTA TTT  
Leu Asn Phe Lys Phe Glu Pro Gln Tyr Lys Asp Val Ser Ala Asn Lys Gin Gly Val Phe  
  
 781 TAT GCA AAA AAC ACC AGT GGA AGC CTT ACT TAT AAA TTA ACG AAA AAA GCA GAC GCT TCT  
Tyr Ala Lys Asn Thr Ser Gly Ser Leu Thr Tyr Lys Leu Thr Lys Lys Ala Asp Ala Ser  
  
 841 GCT ATT CCA CTG AAT GAA TTT GTC AAA CTT ATT GTT GAG GAT AAA GTT AAT ATA CAT ACA  
Ala Ile Pro Leu Asn Glu Phe Val Lys Leu Ile Val Glu Asp Lys Val Ans Ile His Thr  
  
 901 GGG AAT ACT ATT CCA TTA TTA ACC CTT CAA AAG GGA GAC GGG AAA ATT GCT ACT GGT  
Gly Asn Thr Ile Pro Leu Leu Thr Leu Gln Lys Gly Asp Gly Lys Ile Ala Thr Gly  
fanG →  
 961 AAA ATA GAA ACA TTC CTG AAT GTC ACA ATG GAA CAT ATG TGATGAAAAAATTATATAAGCAATA  
Lys Ile Glu Thr Phe Leu Asn Val Thr Met Glu His Met \*\*\*  
MetLysLysLeuTyrLysAlaIle  
 ACAGTCATATGTATATTAATGTCAAATCTACAGTCAGCTCAGGGAGCGACTAACGTCAGTTCAAGGTTCCAATAAGAACAA  
ThrValIleCysIleLeuMetSerAsnLeuGlnSerAlaGlnGlyAlaThrLysSerValGlnValProIleArgThr  
Nsp7524I  
GAGGTTAACGATTCCCTACA  
GluValLysIleProThr

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 promotor 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.*<sup>10)</sup>.

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