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HOKKAIDO UNIVERSITY
CONFIRMED NUCLEOTIDE SEQUENCE OF \textit{fanF} OF \textit{ESCHERICHIA COLI} K99 FIMBRIAE

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Key words: Enterotoxigenic \textit{Escherichia coli}, K99 fimbriae, \textit{fanF} DNA sequence

Enterotoxigenic \textit{Escherichia coli} possessing K99 fimbriae cause diarrhea in newborn calves, piglets and lambs\(^9\). These fimbriae have been found to bind specifically to N-glycolylneuraminic acid-containing GM\(_3\) ganglioside\(^1\) and to consist of eight different subunits named FanA to FanH\(^3\). The nucleotide sequences and functions of these subunits were reported as follows; FanC is a major subunit called fimbrillin which forms the fimbrial structure\(^4\), FanA and FanB are regulatory proteins which control expression of fimbriae\(^5,8\), FanD is platform protein\(^7\), and FanG and FanH are minor subunits\(^6\). However, there is no available information about adhesin, which recognizes the host receptor ganglioside.

Recently, the nucleotide sequences of \textit{fanF}, the gene encoding FanF, were reported by two different laboratories\(^2,10\), but a great difference was observed between nucleotides 769 and the 3' end of the sequences of \textit{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 \textit{et al.}\(^10\) and 813 bp by Ono \textit{et al.}\(^2\). Therefore, we confirmed the nucleotide sequence of this region.

pFK99 (pBR322), which contains the entire K99 fimbrial gene cluster (\textit{fanA} to \textit{fanH})\(^11\) was kindly supplied by Dr. F. K. de Graaf, Vrije University, Amsterdam, the Netherlands. The \textit{BamHI-BamHI} fragment of pFK99 (pBR322), which contains the entire gene cluster, was cloned into pCU19 vector (pFK99 (pUC19)). Then the \textit{NheI-Nsp}\(_{75241}\) fragment of pFK99 (pUC19), which contains the disputed region, was isolated by agarose gel electrophoresis and inserted into pUC18 cleaved with \textit{XbaI} and \textit{SphI}. DNA sequences were determined using a SEQUENASE Ver. 2.0 kit (TOY OBO Co. Ltd., Osaka, Japan).

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

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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.\textsuperscript{10}.

REFERENCES