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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\textsuperscript{9}). These fimbriae have been found to bind specifically to N-glycolylneuraminic acid-containing GM\textsubscript{3} ganglioside\textsuperscript{13} and to consist of eight different subunits named FanA to FanH\textsuperscript{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\textsuperscript{4}), FanA and FanB are regulatory proteins which control expression of fimbriae\textsuperscript{5,8), FanD is platform protein\textsuperscript{7), and FanG and FanH are minor subunits\textsuperscript{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\textsuperscript{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.}\textsuperscript{10) and 813 bp by Ono \textit{et al.}\textsuperscript{2)}. Therefore, we confirmed the nucleotide sequence of this region.

pFK99 (pBR322), which contains the entire K99 fimbrial gene cluster (\textit{fanA to fanH})\textsuperscript{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 \textit{NheI}-Nsp\textsubscript{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 (TOYOBO 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.}\textsuperscript{2)}, \textit{fanF} encoded 333 amino acids. This confirmed nucleotide
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}.

\textbf{REFERENCES}


