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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 lambs⁹. These fimbriae have been found to bind specifically to N-glycolylneuraminic acid-containing GM₃ ganglioside¹ 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 fimbriae^{5,8}, 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 laboratories^{2,10}, 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 *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.*². *fanF* encoded 333 amino acids. This confirmed nucleotide

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sequence was the same as that reported by Simons *et al.*¹⁰⁾.

REFERENCES

- 1) ONO, E., ABE, K., NAKAZAWA, M., & NAIKI, M. (1989): Ganglioside epitope recognized by K99 fimbriae from enterotoxigenic *Escherichia coli*. *Infect. Immun.*, **57**, 907–911.
- 2) ONO, E., LAVIN, M. F., & NAIKI, M. (1991): The nucleotide sequence of the genes, *fanE* and *fanF*, of *Escherichia coli* K99 fimbriae. *Jpn. J. Vet. Res.*, **39**, 1–10.
- 3) OUDEGA, B. & DE GRAAF, F. K. (1988): Genetics organization and biosynthesis of adhesive fimbriae of *Escherichia coli*. *Antonie van Leeuwenhoek*, **54**, 285–299.
- 4) ROSENDAAL, E., GAASTRA, W., & DE GRAAF, F. K. (1984): The nucleotide sequence of the gene encoding the K99 subunit of enterotoxigenic *Escherichia coli*. *FEMS Microbiol. Lett.* **22**, 253–258.
- 5) ROSENDAAL, E., BOOTA, M., & DE GRAAF, F. K. (1987): Two novel genes, *fanA* and *fanB*, involved in the biogenesis of K99 fimbriae. *Nucleic Acids Res.* **15**, 5973–5984.
- 6) ROSENDAAL, E., JACOBS, A. A. C., RATHMAN, P., SONDERMEYER, C., STEGEHUIS, F., OUDEGA, B., & DE GRAAF, F. K. (1987): Primary structure and subcellular localization of two fimbrial subunit-like proteins involved in the biosynthesis of K99 fimbriae. *Mol. Microbiol.* **1**, 211–217.
- 7) ROSENDAAL, E., & DE GRAAF, F. K. (1989): The nucleotide sequence of the *fanD* gene encoding the large outer membrane protein involved in the biosynthesis of K99 fimbriae. *Nucleic Acids Res.* **17**, 1263.
- 8) ROSENDAAL, E., DAMOISEAUX, J., JORDI, W., & DE GRAAF, F. K. (1989): Transcriptional organization of the DNA region controlling expression of the K99 gene cluster. *Mol. Gen. Genet.* **215**, 250–256.
- 9) RUNNELS, P. L., MOON, H. W., & SCHNEIDER, R. A. (1980): Development of resistance with host age to adhesion of K99⁺ *Escherichia coli* to isolated intestinal epithelial cells. *Infect. Immun.*, **28**, 298–300.
- 10) SIMONS, B. L., WILLEMSSEN, P. T. J., BAKKER, D., ROSENDAAL, B., DE GRAAF, F. K., & OUDEGA, B. (1990): Structure, localization and function of FanF, a minor component of K99 fibrillae of enterotoxigenic *Escherichia coli*. *Mol. Microbiol.*, **4**, 2041–2050.
- 11) VAN EMBDEN, J. D. A., DE GRAAF, F. K., SCHOULS L. M. & TEPPEMA, J. S. (1980): Cloning and expression of a deoxyribonucleic acid fragment that encodes for the adhesive antigen K99. *Infect. Immun.*, **29**, 1125–1133.