

豚由来大腸菌から検出された易熱性毒素および薬剤耐性を同時に支配するプラスミド

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—NOTE—

Naturally Occurring Plasmid Coding for Heat-Labile Enterotoxin Production and Drug Resistance from *Escherichia coli* Strain of Porcine Origin

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ABSTRACT. A large plasmid of approximately 120 megadaltons (Md) in size isolated from an enterotoxigenic strain of *Escherichia coli* of porcine origin was found to carry the genes for heat-labile enterotoxin production and drug resistance against tetracycline and kanamycin. This plasmid was non-conjugative itself, but was transmissible to other recipient organisms such as *E. coli* K-12 and *Citrobacter freundii* strains simultaneously in the presence of a 60-Md plasmid.—**KEY WORDS:** heat-labile enterotoxin, plasmid.

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Enterotoxigenic *Escherichia coli* causes diarrheal disease by producing two different toxins, heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST), the production of which is encoded by a plasmid termed Ent [5, 12]. Enterotoxigenic strains showing multiple resistance to various antibiotics have also been isolated [4, 16]. Since some antibiotic-resistance genes are movable from genome to genome by illegitimate recombination events called transposition [2, 8], it is possible that a single plasmid coding for enterotoxin production and drug resistance will be formed in bacterial strains [6, 11, 13].

This report deals with the isolation of a plasmid which carries genes for LT production and resistance to tetracycline (Tc^r) and kanamycin (Km^r).

An enterotoxigenic *E. coli* strain 10 (O:147 K:88⁻) employed in this study was isolated from a 3-day-old piglet which had died from neonatal colibacillosis in Toyama Prefecture, Japan in 1983. LT was detected by using a test kit for Cholera-*Escherichia coli* enterotoxins (Seiken) [1, 3]. ST production was examined by the suckling mouse assay of Takeda *et al.* [14]. Drug susceptibility was

determined by the agar dilution method [10]. Transmissibility of LT production and character of drug resistance were examined by the mixed culture method [15]. *E. coli* K-12 strains, ML 1410 (Met⁻, resistance to nalidixic acid (Nal^r)), C 600 (Lac⁻, Nal^r), C 600 RfP^r (Lac⁻, resistance to rifampicin (Rfp^r)), and *Citrobacter freundii* strain GN 587 (Km^r, Rfp^r) were used as recipients for the mating experiments. Plasmid deoxyribonucleic acid (DNA) was isolated by the method of Kado and Liu [7] and detected by agarose gel electrophoresis [9].

Table 1 shows the properties of various transconjugants and strains cured of their plasmids. The phenotype of the strain 10 was LT⁺, ST⁻ Tc^r Km^r. This strain possessed five different plasmids from approximately 20 to 120 Md in molecular weight. Transconjugants, 1025, 1025 C-3, and D-1, were obtained from mixed cultures using *E. coli* K-12 derivatives as recipients of plasmids. Strain 10 and these transconjugants had a phenotype (LT⁺, Tc^r Km^r) and contained two common plasmids, 60 and 120 Md in size. Strain 1025 H-2 was obtained from strain 1025 C-3 eliminating a 120-Md plasmid by treatment at 42°C

Table 1. Properties of *Escherichia coli* K-12 and *Citrobacter freundii* GN 587 strain transconjugants and their curing strains of plasmids

Strain	Source	Phenotype ^{a)}	Plasmid content (Md)
10	Field strain	LT ⁺ Tc ^r Km ^r	120 110 60 40 20
1025	<i>E. coli</i> ML 1410 transconjugant of 10	LT ⁺ Tc ^r Km ^r	120 110 60
1025 C-3	<i>E. coli</i> C600 Rfp transconjugant of 1025	LT ⁺ Tc ^r Km ^r	120 60
1025 D-1	<i>E. coli</i> C600 transconjugant of 1025 C-3	LT ⁺ Tc ^r Km ^r	120 60
1025 H-2	Curing strain of 1025 C-3	LT ⁻ Tc ^s Km ^s	60
1025 CF 22	<i>C. freundii</i> GN 587 transconjugant of 1025 D-1	LT ⁺ Tc ^r Km ^r	120 60
1025 CF H-1	Curing strain of 1025 CF 22	LT ⁺ Tc ^r Km ^r	120
1025 CF H-2	Curing strain of 1025 CF 22	LT ⁻ Tc ^s Km ^r	None

a) LT; producing heat-labile enterotoxin,

Tc; tetracycline, Km; kanamycin, r; resistance, s; sensitive, +; positive, -; negative.

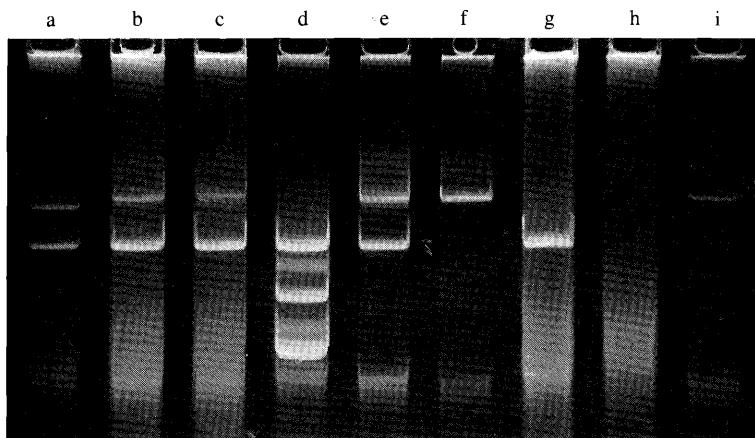


Fig. 1. Agarose gel electrophoresis plasmid DNAs from *Escherichia coli* K-12 and *Citrobacter freundii* GN 587 transconjugants and their curing strains.

Strain 1025 (a), C-3 (b), D-1 (c), CF 22 (e), and H-1 (f) had the same phenotype (LT⁺ Tc^rKm^r). Strain 1025 H-2 (g) was LT⁻ Tc^sKm^s. Strain 1025 CF H-2 (h) was LT⁻ Tc^sKm^r. d (23 Md, 36 Md and 60 Md) and i (120 Md) were indicator plasmid DNAs.

for 3 days. This strain possessed a 60-Md plasmid only, but had lost the characteristics of LT production and resistance to Tc and Km (LT⁻ Tc^s Km^s).

These results suggests that the LT⁺ Tc^r Km^r might be controlled by the 120-Md plasmid but not by the 60-Md plasmid. It was, however, impossible to obtain *E. coli* K-12 strains possessing only the 120-Md plasmid by conjugation.

Next, an attempt was made to produce a strain possessing only the 120-Md plasmid by

using organisms other than *E. coli* as hosts. *Citrobacter freundii* GN 587 strain was chosen a new host organism for the following reasons. Strain GN 587 contained no plasmid and this strain did not produce LT but some *C. freundii* strains were able to produce LT.

Strain 1025 CF 22 was obtained as a *C. freundii* GN 587 transconjugant of strain D-1, at a frequency of 10⁻⁷ per donor cell after 2 hr of conjugation. This transconjugant had two plasmids 60 and 120 Md in size like those possessed by the donor strain, and its pheno-

type was $LT^+ Tc^r Km^r$. However, since strain GN 587 was used as a recipient, which possessed the property of Km^r naturally, it was impossible to determine whether the property of Km^r in strain CF 22 was caused by a plasmid or not. However, when a retransmission experiment was carried out using strain CF 22, C 600 transconjugants were obtained, restoring the property of $LT^+ Tc^r Km^r$. These transconjugants were obtained at a frequency of 10^{-7} after 2 hr of conjugation.

These results indicated that the 120-Md plasmid might also control the appearance of the $LT^+ Tc^r Km^r$ property in the *C. freundii* strain.

Strain CF H-1 and CF H-2 were obtained from strain CF 22 by cultivation at 42°C for 3 days. Strain CF H-1 retained the 120-Md plasmid alone and its phenotype was $LT^+ Tc^r Km^r$. However, when a mating experiment was carried out using strain CF H-1 as a donor, a transconjugant was not obtained by conjugation at either 25° or 37°C for 24 hr with C 600 or ML 1410 as a recipient. It was therefore thought that, this 120-Md plasmid might be non-conjugative. Strain CF H-2 was cured of the 120-Md plasmid and showed a $LT^- Tc^s Km^r$ phenotype.

Fig. 1 shows an electrophoretic picture of plasmid DNA obtained from these strains.

These results indicated that only one 120-Md plasmid carried genes which conferred the property of $LT^+ Tc^r Km^r$ on the host organisms. It was especially interesting that the property of LT^+ controlled by this plasmid was able to be revealed in *C. freundii* strain GN 587. This plasmid was non-conjugative, but it was transmissible to other recipient organisms such as *E. coli* K-12 and *C. freundii* strain simultaneously in the presence of the 60-Md plasmid.

Because of the existence of the 120- and 60-Md plasmids, it is suggested that the use of antibiotics may not only have the effect of

increasing the number of drug-resistant strains but also increasing strains which carry the plasmid coding for enterotoxin production or other pathogenic properties.

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要 約

豚由来大腸菌から検出された易熱性毒素および薬剤耐性を同時に支配するプラスミド (短報): 大前憲一・浜本修一・米沢昭一 (農林水産省動物医薬品検査所)——仔豚由来大腸菌から検出された 120 Md プラスミドは、易熱性毒素産生およびテトラサイクリン・カナマイシン耐性遺伝子群を同時に保有していた。本プラスミドは、自己伝達能を欠いていたが、同一株中に共存していた 60 Md プラスミドと共に他の *E. coli* K-12 株および *C. freundii* 株へ接合伝達が可能であった。