In Vivo Conversion of Transferable Plasmid into R Plasmid in the Intestine of Gnotobiotic Mice

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ABSTRACT. A drug-susceptible strain of mouse Escherichia coli, E916, harboring a transferable plasmid, and a laboratory strain carrying a nonconjugative plasmid, pMK1::Tn2602 (ColE1::Tn5::Tn2602), were orally inoculated into germ-free mice. After administration of kanamycin (Km) in drinking water, Km-resistant (Km') E916 became dominant in the population of fecal flora. Plasmid DNA analysis revealed that Km' E916 possessed a conjugative R plasmid consisting of a transferable plasmid and a Km-transposon (Tn5). The result indicates that the transferable plasmid was converted into an R plasmid while migrating between organisms in the intestine of gnotobiotic mice. —KEY WORD: R plasmid.

Laboratory animals are usually free of drug-resistant organisms because of restrictions on the usage of antimicrobial agents on research animals [3]. In a few animal colonies in which the routine administration of antibiotics had been undertaken, however, high isolation frequencies of drug-resistant Escherichia coli have been observed [8]. The most drug-resistant E. coli isolated from mice and rats have been shown to carry conjugative R plasmids, suggesting that R plasmids play a major role in the emergence of drug-resistant organisms in laboratory animals as they do in humans and domestic animals.

On the other hand, our previous report [7] revealed that about 80% of drug-susceptible E. coli strains isolated from laboratory animals, harbored 1 to 9 cryptic plasmids, one of which was demonstrated to be transferable and to be able to convert into a conjugative R plasmid by translocation of drug resistance transposons in vitro. These facts indicate the possibility that transferable plasmids of drug-sensitive E. coli strains could evolve into R plasmids by the possession of drug resistance genes such as transposons. This paper deals with the possible convertibility of such transferable plasmids into conjugative R plasmids by translocation of drug resistance transposons in the gut of gnotobiotic mice.

E. coli strains used in this experiment were a drug-sensitive mouse strain, E916, harboring 5 plasmid DNAs, one of which (pK0916) was demonstrated to be transferable [7], and E. coli C (a rifampcin-resistant, F" derivative) carrying a nonconjugative plasmid, pMK1 :: Tn2602 (ColE1 :: Tn5 :: Tn2602), which contained a kanamycin (Kn)-transposon (Tn5) and an ampicillin (Ap)-transposon (Tn2602) [10]. Female germ-free mice were obtained commercially from CLEA Japan Inc. The mice kept in an isolator sterilized with 2% peracetic acid and fed on commercial pellets irradiated with 5 Mrad of gamma-rays (Funabashi Farm). Five germ-free mice received 0.1 ml of bacterial culture via a stomach tube (Fig. 1). Fecal samples were periodically collected from each of the mice, suspended in phosphate-buffered saline, and plated on DHL agar (Eiken Chemical Co., Ltd.) containing relevant drugs after serial dilutions. About 100
colonies on agar plates were picked up and assayed for their drug resistance.

As shown in Fig. 1, the average number of C (pMK1::Tn2602) reached a level of $10^6$ per gram of feces on day 1 after injection. After inoculation of E916 on day 3, however, C (pMK1::Tn2602) rapidly decreased to below $10^5$ per gram of feces. E916 always predominated in the population of fecal flora. No drug-resistant organisms were detectable in fecal samples in the absence of antibiotic pressure. After administration of Km (50 μg/ml) in drinking water on day 23, C (pMK1::Tn2602) increased to the level of $10^7$ per gram of feces, and thereafter, Km-resistant (Km') E916 appeared and became dominant at the same level of parental E916. Km'Ap'E916 also increased to a level of $10^7$ per gram of feces on day 31, but did not come to dominate the population of fecal flora.

Km'E916 possessed similar number and size of plasmid DNA bands to parental E916 (data not shown). Then to determine whether the Km-resistance of this organisms depended on transferable R plasmids, we performed a conjugation experiment. A mixed culture of Km'E916 as donor and ML1410 (a nalidixic acid-resistant, methionine-requiring F- derivative of K-12) as the recipient produced the Km' transconjugant, Km'ML1410. Analytical agarose gel electrophoresis revealed that Km'ML1410 contained a single conjugative R plasmid, designated pK0916KmGF, the molecular weight of which was the same as that of another Km resistance plasmid, pK0916-Km, which was obtained from an in vitro conversion experiment and estimated to be 29.5 megadaltons (Mdal) in size (Fig. 2) [7]. Since our previous report [7] had demonstrated that pK0916Km was a composite plasmid consisting of a 26 Mdal transferable plasmid, pK0916, and a Tn5 molecule, pK0916KmGF also appeared to have originated from pK0916 (Fig. 2, arrow) and Tn5. Therefore, pK0916KmGF and pK0916Km were digested with HindIII, BamHI, and EcoRI restriction endonucleases (Takara Shuzo Co., Ltd.) (Fig. 3). Their restriction cleavage patterns were much the same as each other, except that the second HindIII fragment of pK0916KmGF was slightly larger than that of pK0916Km. Small faint fragments in pK0916Km digested with HindIII and BamHI seemed to be originated from pK0916 containing 2 molecules of Tn5, small amount of which always contaminated in pK0916Km preparation. This result suggested that pK0916KmGF was a combined plasmid of pK0916 and Tn5.

Transfer of drug resistance of Km'Ap'E916 was not detected in a conjugation experiment with ML1410 as the recipient. Analytical gel electrophoresis revealed that the transferable plasmid, pK0916, had disappeared, but that two plasmid DNAs of approximately 37 Mdal and 11 Mdal in size had newly appeared in Km'Ap'E916 (Fig. 2, lane E). From their molecular weights, they seemed to be a recombinant plasmid of pK0916 and pMK1::Tn2602 (37 Mdal), and pMK1::Tn2602 itself (11 Mdal), respectively.

Conversion of transferable plasmid into R plasmid occurred in the intestine of gnotobiotic mice. The pK0916 seemed to migrate from E916 to C (pMK1::Tn2602), and change into the R plasmid by translocation of Tn5.
from pMK1::Tn2602 to pKO916. Thereafter, the converted R plasmid, pKO916KmGF, must have returned to E916, and the resultant Km\'E916 increased in the population of fecal flora under the selection pressure of Km. However, Km\'E916 was not detected before administration of Km in drinking water, indicating that the selection pressure of antibiotics was essential for the distribution of drug-resistant organisms and R plasmids in the alimentary tract.

Smith [9] and Anderson [1] described that R plasmid-positive strains of *E. coli* were poorer than their R plasmid-negative counterparts at colonizing the alimentary canal, suggesting that the possession of R plasmid might have a wrong effect on colonization of *E. coli* in the intestine. Although the ability of *E. coli* containing R plasmid to persist in the gut is affected by many factors such as bacterial serotype [2, 6] and age of the animal [4, 5], the relation between the host organisms and plasmid DNAs may also be important, since the majority of drug-sensitive strains of *E. coli* which formed major component of bacterial flora carried plural cryptic plasmids [7]. Our result shows that whereas Km\'E916 possessing pKO916KmGF which was originated from endogenous plasmid, pKO916, could become dominant in fecal flora, Km\'Ap\'E916 carrying the exogenous plasmid, pMK1::Tn2602, and the recombinant plasmid of pMK1::Tn2602 and pKO916 only increased to the level of $10^7$ per gram of feces after the use of Km. From these observations, it seems that the possession of a converted R plasmid by the original host strain of *E. coli* may minimize the decline of the ability to colinize in the alimentary tract.
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REFERENCES


要約

ノトバイオートマス腸管内におけるcrypticプラスミドのRプラスミド化について（短報）：下田耕治・前島一実・寺門誠政11（慶応義塾大学医学部実験動物センター，11農林水産省家畜衛生試験場）——伝達性crypticプラスミドを保有するマウス由来薬剤感受性大腸菌E916および非伝達性プラスミドColE1：Tn5：Tn2602を保有する菌株を無菌マウスに経口投与後、カナマイシン（Km）増加飲水を給与したところ、Km耐性E916が飼育内優勢菌種となった。DNA解析の結果、Km耐性E916は伝達性crypticプラスミドとKmtransposon（Tn5）からなるRプラスミドを保有することが明らかとなった。