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DNA Rearrangement of TOL Plasmid in *Pseudomonas putida* PpG1 Harbouring CAM Plasmid

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CAM 플라스미드를 함유하는 *Pseudomonas putida* PpG1 에서 TOL 플라스미드의 DNA 재배열

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The TOL plasmid, pWWO, conjugally transferred from *Pseudomonas putida* mt-2 was dissociated into TOL* and TOL △ in *P. putida* PpG1 carrying CAM plasmid. The TOL* was integrated into the CAM plasmid, and the resulting plasmid was designated as CAM::TOL*. The introduction of NAH plasmid, belonging to Inc P9 incompatibility group, into *P. putida* CST3A carrying CAM::TOL* plasmid and TOL △ plasmid did not affect *m*-toluate catabolism, but resulted in expelling the TOL △ plasmid.

TOL plasmids are a class of large *Pseudomonas* plasmids which carry the genes for the catabolism of toluene, and *m*-and *p*-xylenes, and thus support the growth of host strains on these compounds as sole sources of carbon and energy. A particularly interesting feature is that TOL plasmid undergoes structural change in the plasmid DNA in which large regions carrying structural gene for *m*-toluate degradation are deleted as result of various physiological changes and thus the strains carrying the deleted plasmid lose the ablity to grow on *m*-toluate (1-4).

In a transconjugant (*P. putida*CST3A) resulting from conjugal transfer of the TOL plasmid, pWWO, into *P. putida* PpG1 carrying CAM plasmid, which confers the degradative potential for camphor, we find that TOL plasmid undergoes the structural change. However, the transconjugant retains the ability to grow on the TOL substrate, *m*-toluate.

In this paper, we resolve this conflict by presenting the evidence that *m*-toluate degrading gene fragment of pWWO is present in the CAM plasmid of *P. putida* PpG1.

Key words: *Pseudomonas putida*, CAM::TOL*, DNA rearrangement
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Materials and Methods

Bacterial strain and plasmid

The bacterial strains and their carrying plasmids used in this experiment are shown in Table 1.

Media and cultivation

Basal minimal salt agar medium described previously was used (5). Carbon sources were provided as vapor by placing naphthalene and camphor crystal on inside of petri-dish cover. *m*-Toluate was directly added to basal minimal salt agar medium. Cultures were incubated at 30°C. When required, antibiotics were added to the medium at a concentration of 100 μg/ml for streptomycin and kanamycin sulfate.

Transfer and detection of plasmid

Conjugation was carried out by mixing the saline suspended donor and recipient cells with ratio of 1:10, standing the mixture overnight at 30°C, and plating the mixture on the selective agar media for the selection of transconjugant (6). Plasmids of parent strain

Table 1. The bacterial strains and their degradative plasmids.

Strain	Plasmid	Sources
P. putida mt-2	TOL	ATCC 33015
P. putida PpG1	CAM	ATCC 17453
P. putida KCTC 2405	*alk*	NCIB 9571
P. putida KCTC 2403	NAH	NICB 8859
P. putida CST3A	CAM::TOL*, TOL △	this work
P. putida 3S	CAM::TOL*, NAH	this work
P. putida SK	CAM::TOL*, *alk*	this work
P. putida SK ▲	TOL ▲, *alk*	this work

TOL* represents nonconjugative, excisable segments specifying all activities required for toluene degradation of TOL plasmid.
TOL△is a deleted TOL plasmid remained after excision of TOL* from TOL plasmid.

and transconjugant were detected by the procedure of Kado and Liu (7).

Results

Conjugal transfer of TOL plasmid into *P.putida* PpG1 harbouring CAM plasmid

A streptomycin resistant strain was derived from *P.putida* PpG1 harbouring CAM plasmid by spontaneous mutation. We performed the conjugation experiment in which this streptomycin resistant strain was used as recipient cell and *P. putida* mt-2 carrying TOL plasmid, pWWO, as donor cell. A streptomycin resistant and *m*-toluate utilizing strain was selected as transconjugant and named *P. putida* CST3A. This transconjugant could utilize *m*-toluate or camphor as sole sources of carbon and energy. As shown in Fig. 1, the transconjugant CST3A harboured two plasmids: L(large) and S(small) plasmids. It was assumed that the L plasmid might be CAM plasmid intrinsically existed in *P. putida* PpG1 and the S plasmid might be generated from pWWO after undergoing the same deletion as the pWWO of *P. putida* mt-2 grown on the benzoate medium (2). The fact that *P. putida* CST3A carrying the deleted TOL plasmid retains the capability to grow on *m*-toltuate as a sole carbon source reflects the presence of the gene for the degradation of *m*-toluate, *tol*, on the genome of this strain, probably chromosome or CAM plasmid.

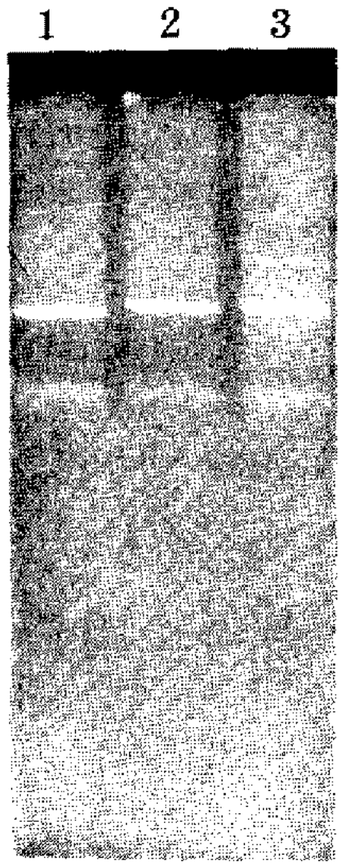

Fig. 1. Agarose gel (0.6%) electrophoretic pattern of plasmids in *P. putida* PpG1 (lane 1), mt-2 (lane 2) and CST3A (lane 3).

Transfer of the degradative potential for camphor and *m*-toluate of *P. putida* CST3A

If *tol* in *P. putida* CST3A existed in the CAM plasmid, it could be cotransferred with CAM plasmid by conjugation. To test this point, we performed the plate mating in which *P. putida* CST3A was used as a donor cell and kanamycin resistant *P. putida* KCTC2405 as a recipient cell. After two strains were mixed, the mixture was plated on the selective media containing kanamycin and camphor (BKC media). The colonies formed on the BKC media were toothpicked onto the media containing kanamycin and *m*-toluate (BKT media). All the bacterial strains (*P.putida* SK) capable of growing on the BKC media were able to grow on the BKT media. This fact indicates that the *tol* in *P. putida* CST3A can be conjugally cotransferred with CAM plasmid into *P. putida* KCTC2405.

On the other hand, when the mixed cell suspension was plated on the BKT media the colonies of kanamycin resistant and *m*-toluate utilizing cells were formed with occurrence of large colonies at the frequency of 10^{-2}, which represents the number of large colony per the number of total colonies formed on the BKT media. The colonies formed on the BKT media except the large colony could grow on the BCT media. The cotransfer of the degradative potential for camphor and *m*-toluate of *P. putida* CST3A reflects

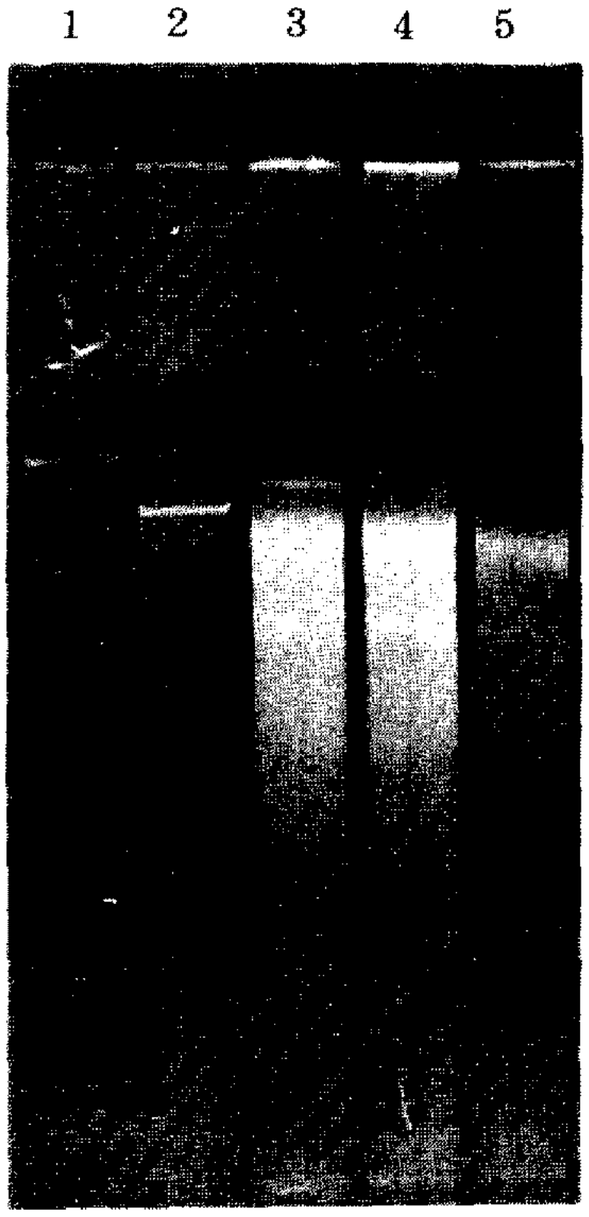

Fig. 2. Agarose gel (0.6%) electrophoretic pattern of plasmids in *P. putida* mt-2 (lane 1), CST3A (lane 2), SK ▲ (lane 3), SK (lane 4) and KCTC 2405 (lane 5).

that *tol* of this strain exists in the conjugative replicon, CAM plasmid. The plasmid for the degradation of camphor and *m*-toluate was designed as CAM::TOL*. The large colony forming strain (*P. putida* SK ▲) on the BKT media harboured a plasmid, which was larger than the deleted TOL plasmid, TOL △, but smaller than the archetype TOL plasmid (Fig. 2). This plasmid might be the replicon for *m*-toluate degradation since it was generated by the conjugation between *P. putida* KCTC2405 harbouring no plasmid, recipient cell, and *P. putida* KCTC2405 harbouring TOL △ and CAM::TOL*, donor cell. This plasmid may be formed by the illegitimate rescue of *tol* gene from CAM::TOL* plasmid by TOL △ during conjugation.

Transfer of NAH plasmid into *P. putida*CST3A

The NAH plasmid of *P. putida* KCTC2403 and the TOL plasmid of *P. putida* mt-2 belong to the same incompatibility group, Inc P9(8). Two plasmids of same incompatibility group can not coexist in a strain. If the gene for *m*-toluate degradation was integrated into the CAM plasmid which belongs to Inc P2 incompatibility group (8), the introduction of NAH plasmid into *P. putida* CST3A did not affect the degradative potential for *m*-toluate of *P. putida* CST3A. Plate mating in which *P. putida* CST3A was used as recipient cell and *P. putida* KCTC2403 containing NAH plasmid as donor cell was performed. The mixture of two strains was plated on the media containing naphthalene as a sole carbon source and streptomycin. A transconjugant *P. putida* 3S was obtained. This transconjugant could utilize naphthalene or *m*-toluate as a sole carbon source and harboured CAM::TOL* and NAH (Fig. 3). The fact that TOL △ plasmid does not exist in *P. putida* 3S indicates that this plasmid encoded the incompatibility system, but did not encode genes for *m*-toluate degradation.

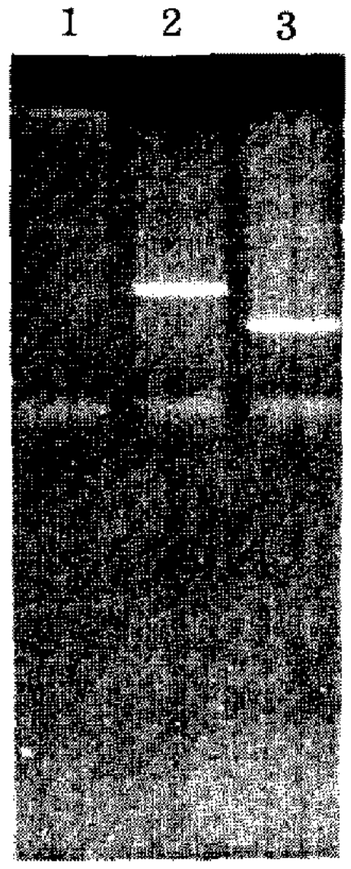

Fig. 3. Agarose gel (0.6%) electrophoretic pattern of plasmids in *P. putida* CST3A (lane 1), KCTC 2403 (lane 2) , and 3S (lane 3).

Discussion

The fate of TOL plasmid in *P. putida* PpG1 carrying CAM plasmid was investigated. The large deletion of TOL plasmid was observed in a transconjugant constructed by conjugal transfer of TOL plasmid into *P. putida* PpG1 carrying CAM plasmid. However, this large deletion does not affect the *m*-toluate catabolism of transconjugant *P. putida* CST3A. The possible location of the genes for *m*-toluate degradation was assumed to be the deleted TOL plasmid, CAM plasmid or chromosome. The deletion, integration and exision of the gene for *m*-toluate degradation of pWWO in a transconjugant constructed by transfer of TOL plasmid into 3-chlorobenzoate-degrading *Pseudomonas* sp. strain B 13 was reported (9, 10). In this experiment, we demonstrated that the genes for *m*-toluate degradation was present in CAM plasmid. Our conclusion

was based on the following evidences, each strongly suggesting plasmid rearrangement. 1) The ability to grow on *m*-toluate could be simultaneously transferred by conjugation with the ability to grow on camphor from *P. putida*CST3A to *P. putida* KCTC2405. 2) Introduction of NAH plasmid, belonging to Inc P9 incompatibility group, into *P. putida* CST3A resulted in expelling the deleted TOL plasmid, but did not affect the *m*-toluate catabolism of *P. putida* CST3A. The possibility that the genes for *m*-toluate degradation were located on the chromosome, and mobilized by CAM plasmid (6), was completely excluded at the basis on the fact that the transfer frequency of CAM phenotype and TOL phenotype was identical.

Chakrabarty *et al.* examined the behavior of TOL in *P. aeruginosa* PAO. TOL frequently underwent a specific dissociation event that resulted in two smaller plasmids designated TOL* and TOL △. The 28 Md nonconjugative TOL* plasmid specified all activities required for the dissimilation of toluene. TOL* was nonconjugative but was mobilized for transfer by K, CAM, and TOL △. TOL* formed 105 Md TOL*-K and 74 kd TOL*-TOL △ cointegrates, no cointegrate was observed between CAM and TOL* in *P. aeruginosa* PAO (1). Our result that CAM and TOL* were cointegratd in *P. putida* PpG1 indicates that cointegration between CAM and TOL* relies on the host strain.

요 약

접합에 의해 *P. putida* mt-2의 TOL를 CAM 함유 *P. putida* PpG1으로 이동시켜 형성된 접합주 *P. putida* CST3A는 작아진 TOL(TOLΔ)를 가지고 있었지만 *m*-toluate를 분해할 수 있었다. 접합에 의한 이동실험과 불화합성 시험결과, TOL의 *m*-toluate 분해유전자는 CAM에 결합되어 CAM : : TOL * 플라스미드를 형성하고 있었다. 불화합성 Inc P9 군에 속하는 NAH를 CAM : : TOL *과 TOLΔ을 가지는 *P. putida* CST3A로 이동시키면 TOLΔ의 방출이 관찰되었으나 *m*-toluate 대사에는 아무런 영향을 미치지 않았다.

References

1. Chakrabarty, A.M., D.A. Friello and L.H. Bopp: *Proc. Natl. Acad. Sci.*, **75**, 3109 (1978).
2. Bayley, S.A., C.J. Duggleby, M.J. Worsey, P.A. Williams, K.G. Hardy and P. Broda: *Mol. Gen. Genet.*, **154**, 203 (977).
3. Downig, R.G., J. Duggleby, R. Villems and P. Broda: *Mol. Gen. Genet.*, **168**, 97 (1979).
4. Williams, P.A., S.D. Taylor and L.E. Gibb: *J. General Microbiol.*, **134**, 2039 (1988).
5. Cho, K.Y., H.K. Chun, K.S. Bae and Y.H. Kho: *Kor. J. Appl. Microbiol. Bioeng.* **16**, 427 (1988).
6. Shaham, M., A.M. Chakrabarty and I.G. Gunsalus: *J. Bacteriol.*, **116**, 944 (1973).
7. Kado, C.I. and S.T. Liu: *J. Bacteriol.* **145**, 1365 (1981).
8. Bagdasariam, M. and K.N. Timmis: *Curr. Topics Microbiol. Immunol* **96**, 47 (1982).
9. Jeenes, D.J., W. Reineke, H.J. Knackmuss and P.A. Williams: *J. Bacteriol.* **150**, 180 (1982).
10. Jeenes, D.J. and P.A. Williams: *J. Bacteriol.* **150**, 188 (1982).

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