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Restriction Map of the R Plasmid pKU10 in *Pseudomonas putida**

Chun, Sung-Hee, Young-Bock Lim, Woong-seop Sim and Yung-Nok Lee
Department of Biology, College of Science, Korea University

*Pseudomonas putida*에 내재하는 Plasmid pKU10의 제한지도

전성희 · 임영복 · 심웅섭 · 이영록
고려대학교 이과대학 생물학과

ABSTRACT: In our laboratory a R plasmid pKU10 was isolated from *Pseudomonas* and its characteristics were investigated. In this study, as a basic work to improve its utility as a cloning vehicle, restriction patterns of pKU10 were analyzed for other various restriction enzymes in addition to restriction endonucleases previously examined. As a result, pKU10 DNA has two cleavage sites for *Cla*I and *Hpa*I, and three sites for *Ava*I. The restriction map of pKU10 was supplemented with *Ava*I, *Cla*I, and *Hpa*I. From the result of this experiment, the usefulness of pKU10 as a cloning vector in *Pseudomonas* will be enhanced by constructions of mini-plasmid or hybrid plasmids.

KEY WORDS □ **Restriction map, R plasmid, pKU10, *Pseudomonas putida***

Gram-negative soil and water bacteria exhibit an enormous range of metabolic activities which are of considerable scientific interest and some of which have potential applications in biotechnology. Certain of these activities cannot be expressed by hybrid *Escherichia coli* strains carrying relevant genetic determinants because of the limited range of metabolic and physiological potentials of this organism. As a result, much effort has been expended to develop versatile host-vector systems that permit DNA manipulations in soil bacteria themselves (Bagdasarian and Timmis, 1982). Vectors for work with *Pseudomonas* are usually based on broad-host-range plasmids belong to incompatibility groups IncP1, IncP4(IncQ) and IncW. These have been recently reviewed by Mermod *et al.* (1986) and Schmidhauser *et al.* (1988). In our laboratory, the naturally occuring IncP-I group plasmid, pKU10, was originally isolated from a *Pseudomonas putida*. The plasmid pKU10 is nonconjugative, but can be mobilized by conjugative SAL plasmids pKU7(Kim *et al.*, 1987). pKU10 is 9.4 Kb in length and confers ampicillin-, tetracyclin-, & chloramphenicol-resistance on its host. It has unique cleavage sites for *Eco*RI, *Xho*I, *Sal*I, *Bgl*II, and *Sma*I(Lim and Lee, 1987).

In this report, as a part of basic work to improve utility of pKU10 as a cloning vehicle, restriction patterns of pKU10 were analyzed for other restriction enzymes in addition to which examined previously. The restriction map of pKU 10 was supplemented.

MATERIALS AND MTHODS

Bacterial Strain and Plasmid

Pseudomonas putida KU816 habouring R plasmid pKU10 was used in this study. Ampicillin, tetracyclin, and chloramphenicol were used at concentrations of 600, 12.5 and 800μg/ml respectively.

Plasmid DNA

Plasmid DNA was prepared with the method of Hansen and Olsen (1978). Plasmid DNA was purified by ultracentrifugation(RP 65T rotor) at 36000 rpm for 48 hr at 18℃ to equilibrium in CsCl-EtBr gradients. Under ultraviolet illumination, plasmid band was collected and extracted three times with water-saturated *n*-butanol, and plasmid solution was dialyzed against several changes of TE buffer for 48 hr.

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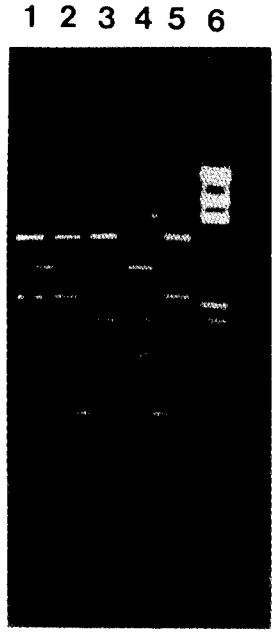

Fig. 1. *Restriction digests of the R plasmid pKU10 with AvaI.*
lane 1 and 5: *Ava*I. Arrow in lane 5 is chromosome.
lane 2: *Ava*I+*Bgl*II. (partial digestion)
lane 3: *Ava*I+*Eco*RI. Arrow is fragment
lane 4: *Ava*I+*Bgl*II+*Eco*RI. Arrow is fragment
lane 6: λ-*Hind*III

Enzymatic Procedure

Restriction enzymes were purchased from Bethesda Research Laboratories and reactions were carried out under the recommended conditions. Enzyme digestions with *Ava*I, *Bam*I, *Bgl*II, and *Eco*RI were carried out in 50 mM Tris-Cl(pH 8.0), 10 mM $MgCl_2$, 50 mM NaCl for 1 hr at 37℃. *Sal*I and *Xba*I conditions were 50 mM Tris-Cl(pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl, and 1 mM dithiothreitol, *Hpa*I and *Cla*I conditions were 10 mM Tris-Cl(pH 7.5), 10 mM $MgCl_2$, and 1 mM dithiothreitol. *Sma*I conditions were 20 mM KCl, 10 mM Tris-Cl(pH 8.0), 10 mM $MgCl_2$ and 1 mM dithiothreitol. DNA fragments were analyzed by 0.7% agarose gel electrophoresis in TAE buffer(Kado and Liu, 1981).

RESULT AND DISCUSSION

In previous report(Lim and Lee, 1987), we described that *Eco*RI, *Xho*I, *Sal*I, *Bgl*II, and *Sma*I cleaved pKU10 once, while *Pst*I cleaved at two sites, and *Hind*III cleaved at six sites. It confers merit as a cloning vector on pKU10 that pKU10

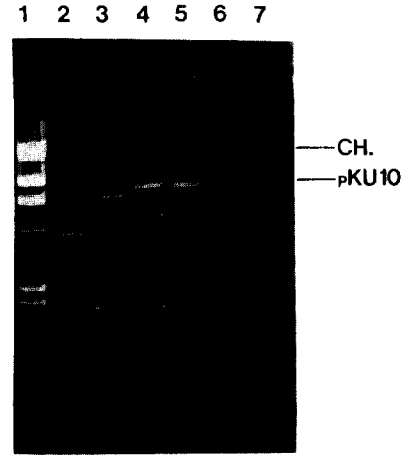

Fig. 2. *Restriction digests of the R plasmid pKU10 with ClaI.*
lane 1: λ-*Hind*III
lane 2: *Cla*I+*Bgl*II+*Eco*RI
lene 3: *Cla*I+*Sal*I+*Eco*RI. Arrow is fragment.
lane 4: *Cla*I+*Sal*I. Arrow is fragment(partial digestion)
lane 5: *Cla*I+*Bal*II (partial digestion)
lane 6: *Cla*I
lane 7: ccc form of pKU10

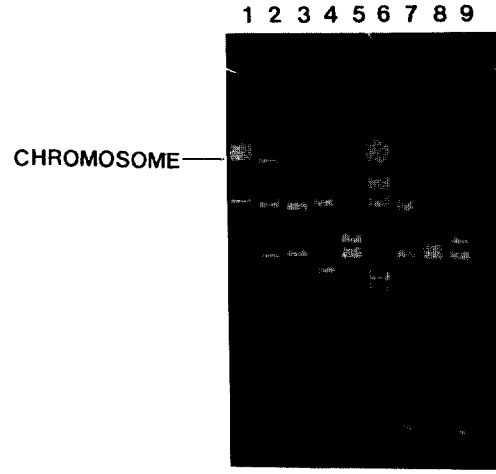

Fig. 3. *Restriction digests of the R plasmid pKU10 with HpaI.*
lane 1: ccc form of pKU10
lane 2: *Hpa*I
lane 3 and 7: *Hpa*I+*Eco*RI
lane 4: *Hpa*I+*Sma*I
lane 5 and 9: *Hpa*I+*Bgl*II
lane 6: λ-*Hind*III
lane 8: *Hpa*I+*Bgl*II+*Eco*RI

Table 1. *Molecular sizes of fragments generated from pKU10 plasmid DNA by various restriction endonucleases used seperately or in combination of two or three.*

Restriction endonucleases	Sizes of the fragments (Kb)* A	B	C	D	Total
*Cal*I	7.5	1.9			9.4
*Cla*I+*Eco*RI	7.4	1.9			9.3***
*Cla*I+*Eco*RI+*Bgl*II	4.3	3.1	1.9		9.3***
*Cla*I+*Eco*RI+*Sal*I	5.7	1.9	1.7		9.3***
*Cla*I+*Sal*I	5.7	1.9	1.8		9.4
*Cla*I+*Bgl*II	4.3	3.2	1.9		9.4
*Cla*I+*Sma*I	7.5	1.0	0.9		9.4
*Ava*I	4.95	2.45	2.0		9.4
*Ava*I+*Eco*RI	4.95	2.0	1.55	0.9	9.4
*Ava*I+*Bgl*II	3.4	2.45	2.0	1.55	9.4
*Ava*I+*Bgl*+*Eco*RI	3.4	2.0	1.55(2)**	0.9	9.4
*Hpa*I	6.5	2.9			9.4
*Hpa*I+*Eco*I	6.0	2.9	0.5		9.4
*Hpa*I+*Sma*I	6.5	2.3	0.6		9.4
*Hpa*I+*Bgl*II	3.6	2.9(2)**			9.4
*Hpa*I+*Bgl*II+*Eco*RI	3.1	2.9(2)**	0.5		9.4

plasmid has unique cloning sites for many of the commonly used restriction endonucleases. However, hybrid plasmids of pKU10 with other more useful genetic markers or miniplasmids of pKU10 should be constructed, because pKU10 plasmids has resistance to antibiotics, such as amplicillin and chloramphenicol, to which some strains of *Pseudomonas* are frequently resistant (Nakazawa, 1983). Accordingly, it is necessary that analysis for other new restriction endonucleases.

As a result of treatment with various restriction endonucleases, *Hpa*I and *Cla*I cleaved pKU10 DNA at two sites and *Ava*I cleaved at three sites, while *Bam*HI and *Xba*I did not cleave. *Ava*I *Cla*I, and *Hpa*I were proved to be useful. The restriction patterns on agarose gel for *Ava*I, *Cla*I, and *Hpa*I are shown in Fig. 1, 2 and 3, respectively. The sizes of fragments generated are shown in Table 1. The locations of the cleavage fragments for three restriction endonucleases on the circular pKU10 map were then found by comparing the fragments with those obtained using each enzyme separately (Fig. 4). The restriction map of pKU10 was supplemented with *Ava*I, *Cla*I, and *Hpa*I.

The wild strains of *Pseudomonas* have naturally resistance to ampicillin and streptomycin (Nakazawa, 1983). The *amp* region of pKU10 located on *Hap*I B(2.9 Kb), *Cla*I B(1.9 Kb), and

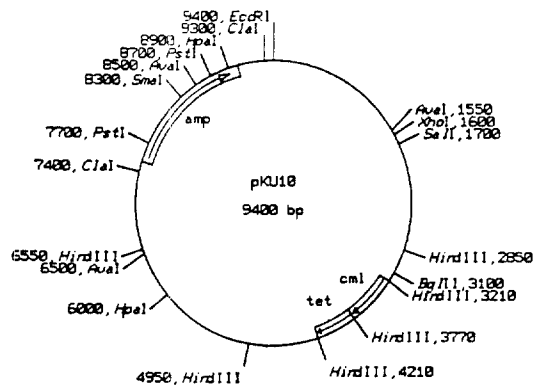

Fig. 4. *Restriction and genetic map of pKU10*

*Ava*I B(2.45 Kb) & C(2.0 Kb) fragment. Therefore, mini-plasmids deleted for relevant fragments to three restrction enzymes are able to be constructed, which have resistance to tetracyclin and chloramphenicol; unvalued *amp* region in *Pseudomonas* will be lost. Another mini-plasmids are able to be self-ligated with fragments digested by the three enzymes. These mini-plasmids will be used to examine the fragment containing replication origin region of pKU10.

적 요

본 연구실에서는 *Pseudomonas* 균주에서 R factor pKU10을 분리하여 그 특성을 조사한 바 있다. 본 연구에서는 이 pKU10을 보다 유용한 vector로 개발하기 위한 기초 작업으로서 이미 조사된 제한효소에 첨가하여 다른 몇가지 제한효소 (*Ava*I, *Cla*I, *Hpa*I)를 처리하여 보완된 pKU10의 제한지도를 작성하였다. 그 결과 pKU10은 *Cla*I 및 *Hpa*I에 의해 두개의

절단부위를 가지며, *Ava*I의 경우 pKU10 DNA를 세군데 자르는 것으로 조사되었다. 이들 결과를 바탕으로 하여 pKU10의 원하는 mini-plasmid를 만들거나, hybrid plasmid를 조립함으로써, 보다 유용한 vector로서의 개발이 가능할 것으로 사료된다.

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