GENERATION OF SMALL PLASMIDS FROM COLICIN E1 FACTOR CARRYING GENES FOR GALACTOSE UTILIZATION AND AMPICILLIN RESISTANCE

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Summary: Ampicillin-resistant colonies that did not utilize galactose appeared sporadically in cultures of galactose gene-deleted Escherichia coli K-12 cells containing colicin E1 factor carrying genes for galactose utilization and ampicillin resistance. Most of these colonies contained small plasmid DNAs. These plasmids existed as monomer DNAs within E. coli K-12 cells and formed a series of covalently closed circular DNA molecules ranging in size from 6.3×10^6 to 15.1×10^6 daltons. The use of these plasmid DNAs was discussed.

Colicin E1 (ColE1) factors that carry lambda phage cohesive end site, genes for galactose utilization and ampicillin resistance have been isolated (1). This paper reports studies on one of these hybrid ColE1 plasmids, named ColE1-amp2113. ColE1-amp2113 DNAs can be introduced into various E. coli K-12 cells by λ phage mediated-specialized transduction, as described for ColE1 carrying genes for guanine synthesis (2). On examining gal-deleted E. coli K-12 cells containing ColE1-amp2113 plasmids, we occasionally found ampicillin resistant colonies that did not utilize galactose. Most of these colonies were found to contain a gene(s) for ColE1 immunity and for ampicillin

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Abbreviations: The genetic symbols are those used by Bachmann et al. (3) for E. coli and by Szybalski and Herskowitz (4) for λ.

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resistance as plasmids. Plasmid DNAs of various sizes (6.3 - 15.1 x 10^6) were obtained from these colonies and five of them were examined by electron microscopy, restriction DNase digestion and agarose gel electrophoresis. The present work showed that this system is useful for isolating closed circular DNAs of various lengths for biological studies and for obtaining potent cloning vehicles.

Materials and Methods

Bacterial and phage strains: *E. coli* K-12 KS1616 is a derivative of HfrH and is deleted of a *gal-attλ-bio* region and a *guaA-guaB* region of a *E. coli* chromosome. The isolation of ColE1-*cosλ-gal* plasmids carrying genes for ampicillin resistance was reported previously (1). ColE1-*amp2113* DNA was transduced into KS1616 by using λ phage (2). Bacteriophage λ*c*I857 and the various λ amber mutants used have been described (2, 5).

Media: Polypeptone bonito extract broth (PBB) medium, PBB agar and MacConkey-galactose agar were described previously (2, 5).

Chemicals: Chloramphenicol was obtained from Yamanouchi Pharmaceutical Co. Ltd., Tokyo and ampicillin from Takeda Chemical Industries, Ltd., Osaka.

Preparation of plasmid DNAs: Various hybrid ColE1 plasmid DNAs were accumulated by incubating cells in PBB in the presence of 100 μg/ml of chloramphenicol. Extrachromosomal DNA was extracted and purified as described previously (6, 7).

Electron microscopic observation of DNA samples: This was performed as described previously (7).

Enzymes: Restriction enzyme *Eco*RI was prepared from *E. coli* strain YR13, which was kindly supplied by Dr. R. Yoshimori (8). *Serratia marescens* endonuclease R (*Sma*R) was purified as described elsewhere (9). Purified plasmid DNAs were digested with restriction enzymes as described elsewhere (7).

Electrophoresis in agarose gel: DNA fragments were subjected to agarose gel (0.8 %) electrophoresis. The gel was stained with ethidium-bromide and fluorescent bands of DNA were photographed under a black light lamp (7).

Detection of residual phage markers: The method of Shimada *et al.* (5) was followed. Other methods of bacterial genetics were as described by Miller (10).

Results and Discussion

E. coli K-12 KS1616 containing ColE1-*amp2113* DNA as plasmid is named *E. coli* KS2113. This strain forms red colonies on MacConkey-galactose agar supplemented with 250

Table 1 Genetic properties of ampicillin resistant mutants not utilizing galactose isolated from KS2113

Strain no.	Name of plasmid	Genetic properties										
		amp^R 1)	ColE1-imm 2)	Phage gene 3) R	A	D	G	J	gal	*4)
KS2113	ColE1-amp2113	+	+	+	+	+	+	+	+	
KS2118	ColE1-amp2118	+	+	+	+	+	+	-	-	1
KS2116	ColE1-amp2116	+	+	+	+	+	-	-	-	2
KS2115	ColE1-amp2115	+	+	+	±	-	-	-	-	1
KS2114	ColE1-amp2114	+	+	+	-	-	-	-	-	5
KS2117	ColE1-amp2117	+	+	-	-	-	-	-	-	4
KS2131	No plasmid	+	-	-	-	-	-	-	-	1

1) Ampicillin resistance was measured as ability to grow on PBB agar plates containing 250 μg/ml ampicillin.
2) The presence of ColE1-imm was tested using colicin E1 and BF23 phage (2).
3) The presence of a given λ phage gene allele in a particular deletion mutant generally resulted in confluent lysis at the location of the spot of λ amber phage (5). ± indicates that only part of this gene is present.
4) * Number of independent isolates.

μg/ml of ampicillin and contains genes for ColE1 immunity and a part of the λ genome, R through J: $(R\text{-}A\text{-}D\text{-}E\text{-}G\text{-}J)^+$(1). White colonies that did not utilize galactose appeared sporadically when we spread single clones of KS2113 on MacConkey-galactose agar containing ampicillin. From the number of white colonies that appeared on a streak on ampicillin containing MacConkey-galactose agar it was estimated that the frequency of cells not utilizing galactose was approximately 10^{-2} to 10^{-4} per viable cell. This frequency is much higher than the mutation frequency.

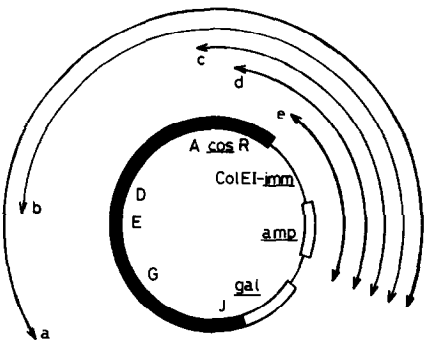

Fig. 1. Structure of small plasmids isolated from ColEl-amp2113. The inner circle represents the proposed structure of ColEl-amp2113 DNA. The thin, single line represents a ColEl DNA, the thick line a part of the λ genome and the double line a fragment of bacterial chromosome. R, A, D, E, G, J and cos are phage markers, ColEl-imm is a ColEl marker. Gal is a bacterial gene and amp indicates a gene(s) for ampicillin resistance. A block of λ genome, N through Q (N-cI-P-Q), is not detected on the ColEl-amp2113 molecule. The set of arcs indicate parts of ColEl-amp2113 DNA covered by various small plasmids. The relative gene order of ColEl-imm and amp is not known from our results. a) ColEl-amp2118, b) ColEl-amp2116, c) ColEl-amp2115, d) ColEl-amp2114 and e) ColEl-amp2117.

We picked up fourteen independent mutants of KS2113 of this type and purified them. Their genetic properties (Table 1) were as follows: 1) One of the fourteen mutants was sensitive to colicin El. The other thirteen were resistant to colicin El and were sensitive to BF23 phage, indicating that they still contained ColEl immunity (2). 2) Nine of thirteen ColEl-imm$^+$ mutants carried various parts of the λ genome. 3) Covalently closed circular DNAs were isolated from ColEl-imm$^+$ strains (see the following section). 4) No covalently closed circular DNAs were found in an extract of chloramphenicol treated KS2131 cells. These results suggest that all the plasmids found in thirteen of the fourteen mutants were derived from the ColEl-amp2113 plasmid by a

Table 2 Molecular nature of newly isolated plasmid DNAs

Name of plasmid	Molecular weight (x 10^6 daltons) determined from electron micrographs[1]	*[2]	Molecular weight (x 10^6 daltons) of DNA fragments produced by SmaR and EcoRI digestion[3]	
			Fragment I	Fragment II
ColE1-amp2118	15.1 ± 0.2	29	4.4	11.0
ColE1-amp2116	12.7 ± 0.3	24	4.4	8.9
ColE1-amp2115	8.8 ± 0.1	30	4.4	4.0
ColE1-amp2114	7.9 ± 0.6	10	4.4	3.6
ColE1-amp2117	6.3 ± 0.2	41	4.4	2.0

1) The length of DNA strands in enlarged photographs was measured with a map measure (7).
2) * Number of open circular DNA molecules measured.
3) The molecular weights of the DNA fragments (I & II) were estimated from Fig. 2 using a reference curve constructed with EcoRI hydrolysates of λ phage DNA (14).

single deletion event including DNA of the galactose operon (Fig. 1).

Structure of newly isolated plasmid DNAs: The plasmid DNAs were accumulated by incubating five of these gal mutants, KS2114 through KS2118, in the presence of chloramphenicol. The DNAs were extracted and the plasmid DNAs were purified by buoyant density centrifugation in ethidium-bromide CsCl (6). The molecular weights of these plasmid DNA molecules were estimated by measuring the contour lengths of the molecules in electron micrographs, relative to that of ColE1 DNA (4.2 x 10^6 daltons)(Table 2). No polymers of these plasmid DNAs were found.

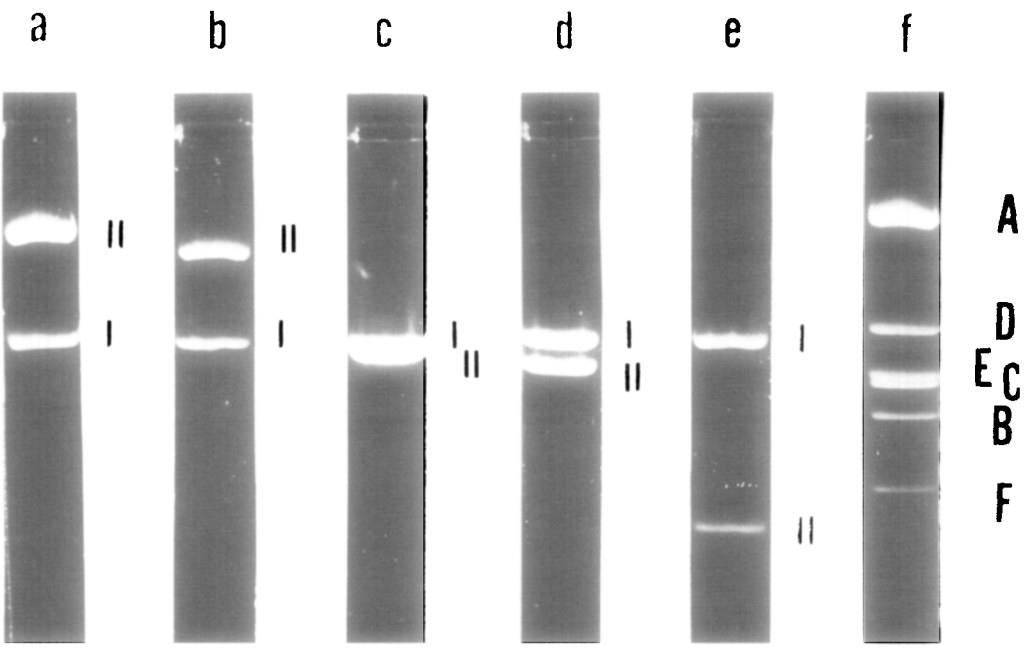

Fig. 2. Agarose gel electrophoresis pattern of digests of newly isolated plasmid DNAs with excess EcoRI and SmaR endonucleases. Samples containing 3 to 5 μg of digested DNA, were applied to 0.8 % agarose slab gel in 40 mM Tris-HCl (pH 7.8), and subjected to electrophoresis at 20 mA/gel for 24 hr at room temperature. Photographs were taken under a black light lamp (Toshiba black light FL 20S·SLB) using Minicopy Film (Fuji) with a Kodak No. 23A red filter. a) ColE1-amp2118, b) ColE1-amp2116, c) ColE1-amp2115, d) ColE1-amp2114, e) ColE1-amp2117, f) λ phage DNA treated with EcoRI. These DNA fragments were used for calibration in determining the molecular weights of the DNA fragments. The molecular weights (x 10^6) of the fragments were as follows: A, 13.7; D, 4.74; E, 3.73; C, 3.48; B, 3.02 and F, 2.18 (14).

The structures of these newly isolated plasmid DNAs were studied by digestion with restriction enzymes and analysis of the resulting fragments by agarose gel electrophoresis. Only single components were isolated after treatment of all these DNAs with EcoRI or SmaR restriction endonuclease (data not shown), indicating that these plasmids have one EcoRI and one SmaR susceptible site, respectively. When the DNAs were

treated with EcoRI and SmaR simultaneously, they produced two DNA fragments (Fig. 2). The sizes of one of the two DNA fragments obtained from these five plasmids were apparently identical (Fig. 2 and Table 2). These results supported the structures presented in Fig. 1.

Potential uses of the plasmid DNAs: The newly isolated plasmids should be convenient sources of covalently closed circular DNAs of assorted sizes for a variety of biological studies. The DNAs range in size from 6.3×10^6 daltons to 15.1×10^6 daltons (Table 2). The ColE1 plasmids are stable and they are easier to grow than most bacteriophage because they can be obtained in stationary cultures and do not require previous viral infection. It is easy to accumulate and isolate ColE1 hybrid DNAs using chloramphenicol treatment (12). Some of the plasmids, i.e. ColE1-amp2115, ColE1-amp2116 and ColE1-amp2118, still carry the cos site of λ phage genome (see Fig. 1). Umene, Shimada & Takagi (manuscript in preparation) found that these plasmids are easily packaged within λ phage particles. The efficient, λ phage-mediated in vivo packaging of small monomer plasmid DNAs with only one cosλ site is an interesting problem, and the plasmid DNAs studied in this work are useful substrates for this study.

Moreover, these newly isolated plasmid DNAs should be potent cloning vehicles for various DNAs. For example, ColE1-amp2115 DNA has a molecular weight of 8.8×10^6 daltons, the ampicillin resistance gene and the replicating machinary of ColE1. These plasmids can be used for cloning of restriction fragments with EcoRI and SmaR termini (7). When a genetically uncharacterized DNA fragment is connected with this ColE1-amp2115 DNA, it can exist as a part of stable plasmid DNA and

can be packaged within λ phage particles, if it is less than 26 x 10^6 daltons in size, because λ phage can package 109 % of the λDNA length (approximately 35 x 10^6 daltons) into its head (2, 13).

The present results on the generation of small plasmids from a ColE1-amp2113 show that one endpoint of the deletions is fixed whereas the other endpoint seems to be at various, but preferred sites (see Fig. 1 & Table 1). We are currently studying the detailed mechanism of deletion formation in this system.

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