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Transposition of the Carbenicillin-Hydrolyzing Beta-Lactamase Gene

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We isolated a new transposon, Tn*2101*, from plasmid Rms433 in *Enterobacter cloacae*. Tn*2101* encoded the formation of type IV (carbenicillin-hydrolyzing) β-lactamase and multiple resistance to streptomycin, sulfanilamide, spectinomycin, and mercury in addition to ampicillin. Tn*2101* was transposable between conjugative (or nonconjugative) plasmids and the host chromosome. Tranposition occurred independently of the general recombination ability of the host cell. Tn*2101* had a molecular size of 9.5×10^6 and contained short inverted repeat terminal sequences.

A number of antibiotic resistance genes on plasmids have been shown to be located in transposable DNA units termed transposons. Transposons can move between different replicons by *recA*-independent recombination systems (15). Plasmid-mediated resistance to penicillins and cephalosporins is mostly due to the formation of β-lactamase. The β-lactamases specified by R plasmids are now classified into four types based on their enzymological and immunological properties (20). Of these enzymes, the type I (or TEM type) β-lactamase is the most common enzyme and is most often produced by plasmids of a variety of incompatibility groups (19). The type II (oxacillin-hydrolyzing, or OXA type), type III (OXA type), and type IV (carbenicillin-hydrolyzing, or PSE type) enzymes are less frequently seen in clinical isolates carrying plasmids (8, 10). Recently, we reported that R-plasmid-mediated type IV β-lactamases have been found in various species of bacteria (*Escherichia coli*, *Proteus mirabilis*, and *Enterobacter cloacae*) and in many plasmids of various incompatibility groups (13). The structural gene encoding the formation of type I (11) β-lactamase mostly resides on discrete DNA sequences capable of *recA*-independent transposition. Moreover, Tn*2603*, a transposon encoding type II β-lactamase, was also reported by Yamamoto et al. (28).

In this paper, we show that the carbenicillin-hydrolyzing β-lactamase (*car*) gene carried by plasmid Rms433 is located on a transposon, called Tn*2101*. This transposon encodes resistance to streptomycin, sulfanilamide, spectinomycin, and mercury in addition to the formation of type IV β-lactamase.

MATERIALS AND METHODS

Bacterial strains and plasmids. Table 1 lists the bacterial strains and plasmids used. All bacterial strains are derivatives of *E. coli* K-12. Both χ490 and χ2206 were gifts from R. Curtiss III (University of Alabama in Birmingham). χ2206 transduced by *polA214* to χ1411 was constructed by M. Inoue and R. Curtiss III (personal communication). Rms433 is a conjugative plasmid originally isolated from *E. cloacae*. A conjugative plasmid, TP114, and two nonconjugative plasmids, pCR1 and pACYC184, were used as acceptors of Tn*2101* from Rms433.

Media and drugs. L broth (17) and BTB lactose agar were routinely used for liquid and plate cultures, respectively. BTB lactose agar consisted of nutrient broth (10 g of beef extract, 10 g of peptone, and 3 g of NaCl in 1,000 ml of distilled water) supplemented with 80 mg of bromothymol blue, 10 g of lactose, and 15 g of agar. Mueller-Hinton medium was used to assay sulfanilamide resistance. Brain heart infusion broth was used for determination of β-lactamase activity. BSG buffer consisted of 8.5 g of NaCl, 300 mg of KH_2PO_4, 100 mg of gelatin, and 1,000 ml of distilled water. Antibiotics used were: ampicillin (Ap; Toyama Chemical Co., Japan), piperacillin (Pip; Toyama Chemical Co.), carbenicillin (Cb; Fujisawa Pharmaceutical Co., Japan), streptomycin (Sm; Toyojozo Co., Japan), sulfanilamide (Sa; Dainippon Pharmaceutical Co., Japan), spectinomycin (Spc; Japan Upjohn Research Laboratories, Japan), kanamycin (Km; Meiji Seika Co., Japan), tetracycline (Tc; Japan Lederle Co., Japan), chloramphenicol (Cm; Toyojozo Co.), nalidixic acid (Na; Daiichi Pharmaceutical Co., Japan), rifampin (Rp; Daiichi Pharmaceutical Co.), and mercuric chloride (Hg; Wako Pure Chemical Industries, Ltd., Japan).

Drug resistance. Drug resistance was determined by the agar dilution method, and the level of drug resistance was expressed as the minimal inhibitory concentration (MIC) of each drug. Plates were inoculated with one loopful (about 5 μl) of 10^4 cells per ml of an overnight culture in L broth. The MIC values were scored after 18 h of incubation at 37°C.

Genetic transfer. Conjugation was carried out in broth (23). Exponential-phase cultures in L broth (about 2×10^8 cells/ml) of donor and recipient were mixed at a volume ratio of 1:10. The mixture was incubated for 2 h at 37°C without aeration, washed

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genetic properties	Source, derivation or reference
E. coli K-12		
χ490	F⁻ λ⁻ *argG28 gal-5 his-4 lac-29 leu-2 malA10 metB6 mtl-2 recA2 rpsL tonA xyl-3*	R. Curtiss III
χ1037	F⁻ *galK2 galT22 hsdR lacY1 metB1 relA supE44*	R. Curtiss III
χ2206	F⁻ λ⁻ *minA minB2 nalA25 polA214*(Ts) *thyA*	Isolated from χ1411 (R. Curtiss III)
ML4901	Na^r mutant of χ1037	
ML4905	Rp^r mutant of χ1037	
Plasmid		
Rms433	Ap Sm Sa Hg Tra^+ low copy	Isolated from *E. cloacae* GN5774
TP114	Km Tra^+ low copy	Via G. Satoh (7)
pCR1	Km Tra^- high copy	E. Lederberg (1) via R. Curtiss III
pACYC184	Tc Cm Tra^- high copy	E. Lederberg (4)

with BSG buffer, diluted appropriately, and then plated on selection media. Transformation was conducted according to the method of Curtiss et al. (5).

The concentrations of the drugs (in micrograms per milliliter) used in selection media were: ampicillin, 25; streptomycin, 12.5; sulfanilamide, 200; kanamycin, 12.5; tetracycline, 12.5; chloramphenicol, 25; nalidixic acid, 25 and rifampin, 100, respectively.

Preparation of plasmid DNA. Bacteria carrying plasmids were grown in 200 ml of L broth at 37°C. The cells at the stationary phase of growth were harvested by centrifugation and washed with 30 ml of 10 mM Tris–1 mM EDTA (pH 8.0). A cleared lysate was then prepared by the Triton X-100 salt precipitation method (16). Plasmid DNA was isolated by ethidium bromide-cesium chloride gradient centrifugation (12).

Restriction endonuclease digestion and analytical gel electrophoresis. Endonuclease *Eco*RI was the product of New England Biolabs. Plasmid DNA digestions were carried out for 2 h at 37°C in 100 mM Tris-hydrochloride (pH 7.5), 5 mM $MgCl_2$, 50 mM NaCl, and 100 μg of bovine serum albumin per ml for 2 h at 37°C. Digestion was stopped by adding blue juice at a fifth part of volume of the reaction mixture. Blue juice consisted of TEAS buffer containing 0.1% bromophenol blue, 60% sucrose, and 20 mM EDTA. TEAS buffer consisted of 50 mM Tris, 20 mM sodium acetate, 2 mM EDTA, and 18 mM NaCl, adjusted to pH 8.0 with glacial acetic acid. The reaction mixtures were subjected to electrophoresis in horizontal slab gels of 0.8% agarose. TEAS buffer was used as a solvent and electrode buffer. Electrophoresis was carried out at 35 mA for 16 h. After electrophoresis, the gels were stained with ethidium bromide and photographed under UV light.

The sizes of DNA fragments were estimated from their mobility in comparison with those of *Eco*RI-digested fragments of R100 DNA. The molecular sizes used for the R100 fragments were 13.5, 8.0, 7.6, 7.2, 5.0, 4.0, 3.5, 3.2, 2.7, 1.79, 1.10, 1.01, and 0.75 megadaltons (Md) (22).

Heteroduplex formation and electron microscopic observation. DNA-DNA heteroduplexes were prepared by the method of Davis et al. (6). The single-stranded form and double-stranded relative form of phage fd DNA with a size of 6.4 kilobases were added as the standards in electron microscopic observation (2). The concentration of formamide in the spreading solution (hyperphase) and the hypophase were 50 and 20%, respectively. The length of the single-stranded DNA was 83% of the corresponding double-stranded DNA under this condition.

Assay of β-lactamase activity. Bacteria were grown overnight at 37°C in 5 ml of brain heart infusion broth. The culture was diluted with 50 ml of fresh brain heart infusion broth and then grown at 37°C with shaking. After 4 h of incubation, the cells were harvested by centrifugation and washed twice with 50 ml of 50 mM phosphate buffer (pH 7.0). The cells were suspended in 5 ml of the same buffer and disrupted by sonic treatment for 3 min. The sonicated extract was centrifuged, and the supernatant was used for the crude enzyme solution. β-Lactamase activity was determined by spectrophotometric assay at 30°C (23).

Determination of protein concentration. The protein concentration in the enzyme solution was estimated by the method of Lowry et al. (18), using bovine serum albumin as the standard.

RESULTS

Transposition of the *car* gene on Rms433. Plasmid Rms433, originally isolated from *E. cloacae*, encodes the formation of a type IV (carbenicillin-hydrolyzing) β-lactamase which gives high rate of hydrolysis of carbenicillin. The isoelectric point of this enzyme is 5.7. We first examined the transposability of the *car* gene on Rms433 to other replicons (Fig. 1). Plasmid Rms433 was conjugally transferred to *E. coli* ML4905 carrying the high-copy-number plasmid pCR1. It has been reported that the resistance level to ampicillin changes proportionally to the dosage of the β-lactamase gene on the plasmid genome (21). *E. coli* ML4905 (Rms433) used in our study was highly resistant to ampicillin (MIC, 400 μg/ml). Piperacillin was more active

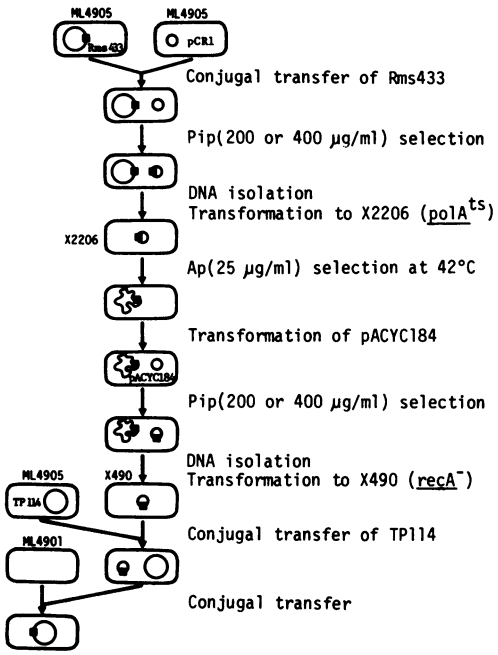

FIG. 1. Transposition procedure of the *car* gene.

than ampicillin against this strain, although it was partially hydrolyzed by the type IV β-lactamase. We noted a correlation between resistance to piperacillin and to ampicillin. Therefore, highly piperacillin-resistant clones should be hyperresistant to ampicillin. A log-phase culture of strain ML4905 (pCR1/Rms433) was plated on BTB agar containing a high concentration of piperacillin (200 or 400 μg/ml) and incubated for 24 h at 30°C. Highly piperacillin-resistant clones were isolated at a frequency of 5×10^{-5} (Table 2). The resistance levels to ampicillin and piperacillin of these clones was 16-fold higher than those of the parent strain, and their β-lactamase activities were proportional to the increased level of resistance to these drugs. In addition, their resistance levels to streptomycin and sulfanilamide were fourfold higher than those of the parent strain (Table 3).

To analyze these clones, the plasmid DNA was prepared and transformed to χ2206. Twenty transformants which acquired ampicillin resistance were randomly chosen from selective plates containing both ampicillin and nalidixic acid, purified, and tested for stability of the plasmid at 30 or 42°C. When the transformants were incubated at 30°C, all could grow on plates containing ampicillin, streptomycin, sulfanilamide, or mercuric chloride and showed high resistance to ampicillin and piperacillin. In contrast, they could not grow on drug-containing plates at 42°C. These results suggested that the Ap Sm Sa Hg genes on Rms433 were transposed to plasmid pCR1. Replication of plasmid pCR1 is dependent on polymerase I activity, i.e., *polA* function; therefore, pCR1 cannot replicate at 42°C in χ2206 which cannot produce polymerase I at 42°C. Thus, the plasmid pCR1::(Ap Sm Sa Hg, designated as Tn*2101*) was lost from the host cell at 42°C.

To isolate a *polA*-independent strain carrying ampicillin resistance, strain χ2206 carrying pCR1::Tn*2101* was incubated on either a drug-free plate or an ampicillin plate at 42°C. Clones which grew on the ampicillin plate at 42°C were obtained at a frequency of 10^{-5}, and all 10 clones tested showed resistance to ampicillin, streptomycin, sulfanilamide, and mercuric chloride. Resistance to these drugs was stable even when the clones were incubated at 42°C. However, the resistance levels to ampicillin and piperacillin and their β-lactamase activities were decreased 20-fold compared with those of the strain carrying pCR1::Tn*2101*, suggesting that Tn*2101* was transposed from pCR1 to the χ2206 chromosome (Table 3).

We transposed Tn*2101* from the χ2206 chromosome to another high-copy-number plasmid. Plasmid pACYC184 was transformed into strain χ2206::Tn*2101*. A transformant was spread on plates containing piperacillin (200 or 400 μg/ml), and clones highly resistant to piperacillin were isolated at a frequency of 3×10^{-5} (Table 2). Plasmid DNA was extracted from each of six strains showing a high level of piperacillin resistance and transformed to χ490. Six strains were

TABLE 2. Transposition frequency of Tn*2101*

Donor	Acceptor of *car* gene	Chromosome marker of host strain	Frequency[a]
Rms433	pCR1	*recA*$^+$	5×10^{-5}
pCR1::Tn*2101*	Chromosome	*polA*(Ts)	1×10^{-5}
Chromosome::Tn*2101*	pACYC184	*polA*(Ts)	3×10^{-5}
pACYC184::Tn*2101*	TP114	*recA*	5×10^{-5}
pACYC184::Tn*2101*	TP114	*recA*$^+$	8×10^{-5}

[a] Expressed as the number of Tn*2101*$^+$ clones containing acceptor marker per number of donor strains or TP114$^+$ transconjugants.

TABLE 3. Drug resistance and β-lactamase activity specified by various plasmids containing Tn*2101*

Strain	Level[a] of resistance (MIC, μg/ml) to:									β-Lactamase activity[b] (U/mg of protein)
	Ap	Pip	Sm	Sa	Spc	Hg	Km	Tc	Cm	
ML4905 (Rms433)	400	50	50	800	200	50	0.8	0.8	1.56	0.53 (1)[c]
ML4905 (pCR1::Tn*2101* Rms433)	>3,200	800	200	3,200	800	100	400	0.8	1.56	5.88 (11.1)
χ2206 (pCR1::Tn*2101*)	>3,200	800	200	3,200	800	100	400	0.8	1.56	5.16 (9.7)
χ2206::Tn*2101*	400	50	50	800	100	50	0.8	0.8	1.56	0.27 (0.5)
χ490 (pACYC184 ::Tn*2101-9*)	>3,200	400	>400	1,600	200	100	0.8	50	6.25	3.30 (6.2)
χ490 (pACYC184 ::Tn*2101-13*)	>3,200	400	>400	1,600	200	100	0.8	0.8	200	3.13 (5.9)
ML4905 (pACYC184 ::Tn*2101-9*)	3,200	800	200	3,200	400	100	0.8	50	12.5	5.71 (10.8)
ML4901 (TP114 ::*Tn2101-9*)	400	50	100	800	200	50	400	0.8	1.56	0.54 (1.0)
ML4905	3.13	1.56	0.8	≦12.5	6.25	1.56	0.8	0.8	1.56	<0.05
χ2206	3.13	1.56	0.8	≦12.5	6.25	1.56	0.8	0.8	1.56	<0.05
χ490	1.56	0.8	>400	≦12.5	3.13	1.56	0.8	0.8	0.8	<0.05
ML4901	3.13	1.56	0.8	≦12.5	6.25	1.56	0.8	0.8	0.8	<0.05

[a] Determined by the agar dilution method and after incubation at 37°C for 18 h.
[b] Crude β-lactamase preparations were used. β-Lactamase activity was assayed by the spectrophotometric method at 30°C (24). Carbenicillin (100 μM) was used as the substrate.
[c] Relative activity is given in parentheses.

independently obtained, and these showed two different types of drug resistance patterns. Four strains carrying pACYC184::Tn*2101-2*, *-3*, *-5*, or *-9*, were resistant to ampicillin, streptomycin, sulfanilamide, mercuric chloride, tetracycline, and chloramphenicol, and two strains carrying pACYC184::Tn*2101-10* and *-13* coded for resistance to all of these antibiotics except tetracycline. Their β-lactamase activities were high in parallel with increased levels of ampicillin resistance. However, the levels of chloramphenicol resistance of the strains carrying pACYC184::Tn*2101-2*, *-3*, *-5*, or *-9* were decreased about 15- to 30-fold compared with that of the strain carrying pACYC184. No differences between pACYC184::Tn*2101-9* and pACYC184::Tn*2101-13* in plasmid copy number were observed, because two strains carrying each plasmid showed the same β-lactamase activity (Table 3). Therefore, the lower level of chloramphenicol resistance might have been due to the lower expression of the chloramphenicol operon conferred by the transposition of Tn*2101* into pACYC184. Based on these results, we concluded that Tn*2101* had been transposed to different sites on plasmid pACYC184. The plasmid DNAs of six strains were prepared and used for agarose gel electrophoresis and for electron microscopic analysis.

We examined whether transposition of Tn*2101* was *rec* independent. Plasmid TP114 was conjugally transferred to χ490 (*rec*) or ML4905 (*rec*$^+$) carrying pACYC184::Tn*2101-9*. Strains carrying both pACYC184::Tn*2101-9* and TP114 were used as the donors for conjugation with ML4901. The nonconjugative plasmid pACYC184 is not mobilized by conjugative drug resistance plasmids such as TP114 (M. Inoue et al., unpublished data). From the donors, plasmid TP114 was transferred at a frequency of about 10^{-2} when selected with kanamycin. Transconjugants which acquired the resistance markers of Tn*2101* were obtained at a frequency of about 10^{-7}, and all 10 transconjugants tested had acquired the kanamycin resistance marker of TP114, but neither the tetracycline nor chloramphenicol marker of pACYC184. The resistance levels to ampicillin, piperacillin, streptomycin, sulfanilamide, and mercuric chloride of the transconjugants were the same as those of strain ML4905 (Rms433) (Table 3). We confirmed that these transconjugants carry TP114::Tn*2101* recombinant plasmids, because resistance markers of Tn*2101* were always trans-

ferred together with TP114 in successive transfers. The transposition frequency in χ490 or ML4905, expressed as the ratio of the number of transconjugants, i.e., TP114::Tn*2101*$^+$ to TP114$^+$, was 5×10^{-5} (*rec*) or 8×10^{-5} (*rec*$^+$), respectively. These results indicate that the transposition of Tn*2101* can occur independently of the *recA* function of the host.

Determination of the molecular size of Tn*2101*. The plasmid DNAs of the six strains carrying pACYC184::Tn*2101* were compared with that of pACYC184 by digestion with the restriction endonuclease *Eco*RI. Gel electrophoresis revealed four different cleavage patterns (Fig. 2). There was a similarity in the cleavage pattern of DNA from recombinant plasmids 2 and 3 (Fig. 2, lanes 2 and 3), and the pattern of recombinant 10 (Fig. 2, lane 6) was similar to that of no. 13 (Fig. 2, lane 7). In all cases, four fragments were generated. As pACYC184 had only one *Eco*RI cleavage site, we concluded that Tn*2101* had three *Eco*RI sites. Of the four fragments, the two fragments of 3.52 and 1.68 Md were common, and the remaining two fragments had different sizes. The latter two included pACYC184 DNA, and the other two fragments were derived from Tn*2101*. The sizes of the two pACYC184::Tn*2101* fusion fragments indicated the relative distance of the insertion sites of Tn*2101* from the *Eco*RI site of pACYC184, thus showing transposition to various sites on pACYC184. The sizes of the fragments were determined from several experiments and are summarized in Table 4. The sum of the fragment sizes was 12.11 Md. Since pACYC184 was 2.65 Md, the difference between these molecules was 9.46 Md (14.2 kilobase pairs long), which corresponded to the molecular size of Tn*2101*.

Observation of Tn*2101* by electron microscopy. Heteroduplex molecules were formed between plasmids pACYC184 and pACYC184::Tn*2101* (Fig. 3). Electron microscopy revealed three kinds of molecules. Two of them were double-stranded DNAs, and the third consisted of heteroduplex molecules between pACYC184 and pACYC184::Tn*2101*-9 DNAs. Heteroduplexes show a single-stranded loop, indicating that the transposition of Tn*2101* occurred as a single contiguous insertion of DNA into pACYC184. In these molecules, the double-stranded region had a molecular size of 2.64 ± 0.02 Md, and the single-stranded region loop was 9.5 ± 0.24 Md; they corresponded to pACYC184 DNA and Tn*2101* DNA, respectively. The heteroduplex

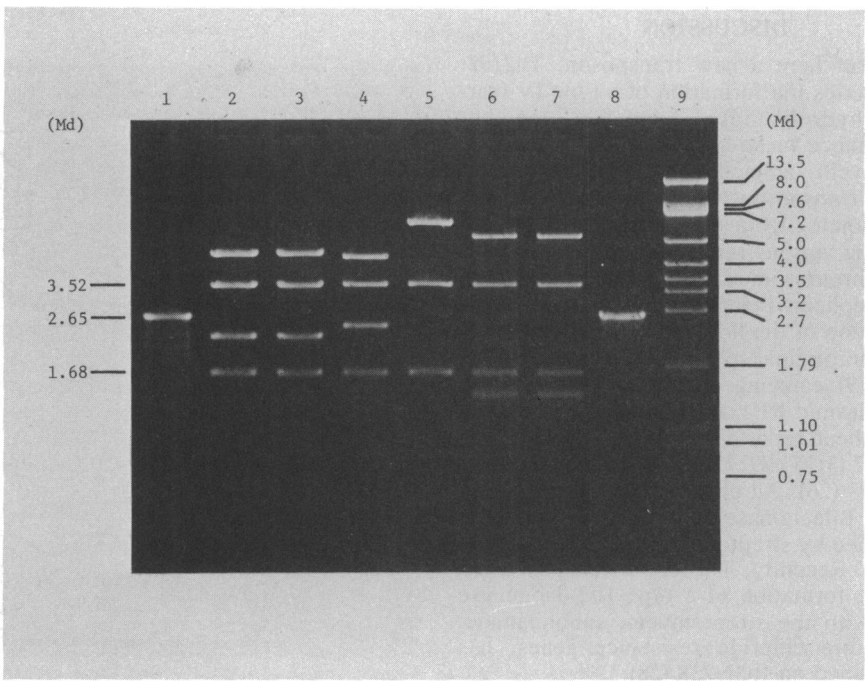

FIG. 2. Agarose gel electrophoresis of pACYC184 and pACYC184::Tn*2101* DNAs cleaved by *Eco*RI. Lanes 1 and 8, pACYC184 DNA; lane 2, pACYC184::Tn*2101-2* DNA; lane 3, pACYC184::Tn*2101-3* DNA; lane 4, pACYC184::Tn*2101-5* DNA; lane 5, pACYC184::Tn*2101-9* DNA; lane 6, pACYC184::Tn*2101-10* DNA; lane 7, pACYC184::Tn*2101-13* DNA; lane 9, reference DNA (*Eco*RI-cleaved R100 DNA). The molecular weights ($\times 10^6$) of the R100 segments were as follows: 13.5; 8.0; 7.6; 7.2; 5.0; 4.0; 3.5; 3.2; 2.7; 1.79; 1.10; 1.01; and 0.75.

TABLE 4. *Eco*RI-cleaved fragments of pACYC184::Tn*2101*

Plasmid	Fragment size (Md)	Total (Md)
pACYC184::Tn*2101-2*	4.66, 3.52, 2.26, 1.68	12.12
pACYC184::Tn*2101-3*	4.66, 3.52, 2.26, 1.68	12.12
pACYC184::Tn*2101-5*	4.42, 3.52, 2.46, 1.68	12.08
pACYC184::Tn*2101-9*	6.40, 3.52, 1.68, 0.54	12.14
pACYC184::Tn*2101-10*	5.53, 3.52, 1.68, 1.39	12.09
pACYC184::Tn*2101-13*	5.53, 3.52, 1.68, 1.39	12.09
pACYC184	2.65	2.65

molecule in the photograph (Fig. 3) contained two stemlike structures indicative of inverted repeat DNA sequences. One internal inverted repeat sequence was at a distance of 4.2 kilobases from the Tn*2101* insertion site and was about 170 base pairs long. The size of the single-stranded DNA loop flanked by this inverted repeat sequence was approximately 900 base pairs. The other was observed at the insertion site of Tn*2101* into pACYC184 and was less than 100 base pairs long. Heteroduplex analysis also indicated that the transposition of Tn*2101* into pACYC184 did not cause any significant deletion.

DISCUSSION

We report here a new transposon, Tn*2101*, which encodes the formation of a type IV (carbenicillin-hydrolyzing) β-lactamase together with resistance to streptomycin, sulfanilamide, spectinomycin, ampicillin, and mercuric chloride. This transposon was identified in plasmid Rms433 isolated from *E. cloacae*. It can transpose among various replicons such as plasmids and host chromosome and to various sites within a single replicon independently of the normal *recA* function of the host cell.

Since the original recognition of the transposon Tn*1* (9) carrying an ampicillin resistance gene on plasmid RP4, many ampicillin transposons have been reported: Tn*2* (11), Tn*3* (15), Tn*4* (14), Tn*401* (3), Tn*902* (29), Tn*1701* (25), Tn*2601*, and Tn*2602* (26). All of these transposons carry the type I β-lactamase gene. Moreover, Tn*4* is accompanied by streptomycin and sulfanilamide resistance. Recently, Tn*2603*, a transposon encoding the formation of a type II β-lactamase together with the streptomycin, sulfanilamide, and mercuric chloride resistance genes, has been identified on RGN238 (28).

Tn*2101* is different from other ampicillin transposons because it encodes a type IV β-lactamase. In addition, the size of Tn*2101* is 9.5 Md, which is somewhat smaller than the sizes of Tn*4* and Tn*2603*, which confer resistance to ampicillin, streptomycin, and sulfanilamide. The sizes of Tn*4* and Tn*2603* have been reported to be 13.6 Md and 19.6 kilobases, respectively (14, 28). Tn*2101* also contains the single-stranded DNA loop flanked by inverted repeat sequences about 170 base pairs long within the molecule, the genetic properties of this locus remain unknown.

Tn*4* includes Tn*3* in its composition, and thus the ampicillin resistance gene is capable of independent transposition. However, it has been reported that transposition of the *oxa* gene of Tn*2603* is always accompanied by streptomycin and sulfanilamide resistance genes. According to their cleavage analysis, Yamamoto et al. (27) have also suggested that Tn*2603* can be constructed by insertion of a DNA segment containing the *oxa* gene into the region of the streptomycin and sulfanilamide resistance genes on Tn*21*, which encodes the streptomycin, sulfanilamide, and mercuric chloride resistance genes. It is not yet known whether the internal stem-loop structure of Tn*2101* can be generated by insertion of the *car* gene into Tn*21*, nor whether the *car* gene of Tn*2101* is transposable among various replicons independently of the other drug resistance determinants.

The transposition frequencies of the known ampicillin transposons specifying type I or II β-lactamase have been reported to be about 10^{-2}

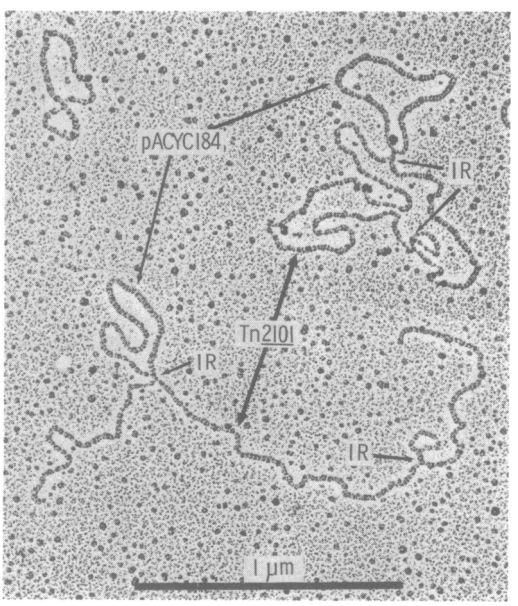

FIG. 3. Heteroduplex between pACYC184 and pACYC184::Tn*2101-9* DNAs. The single-stranded region corresponds to Tn*2101*, which contains two small inverted repeat (IR) sequences. In one of the heteroduplex molecules shown, the single-stranded DNA loop (Tn*2101*) is broken.

to 10^{-4} (26) or about 10^{-3} to 10^{-4} (28), respectively. The transposition frequency of Tn*2101* is lower (about 10^{-5}) than those of the other ampicillin transposons. The type I β-lactamase occurs most frequently and with the widest distribution. The type II and III enzymes are less prevalent. The type IV enzyme is frequently found in the *Pseudomonas* group of organisms. The limited distribution of the type IV β-lactamase among microorganisms may be caused by the low transposability of the *car* gene.

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LITERATURE CITED

1. **Armstrong, K. A., V. Hershfield, and D. R. Helinski.** 1977. Gene cloning and containments properties of plasmid ColE1 and its derivatives. Science **196**:172–174.
2. **Beck, E., R. Sommer, E. A. Auerswald, C. Kurz, B. Zink, G. Osterburg, H. Shaller, K. Sugimoto, H. Sugisaki, T. Okamoto, and M. Takanami.** 1978. Nucleotide sequence of bacteriophage fd DNA. Nucleic Acids Res. **5**:4495–4503.
3. **Benedick, M., M. Fennewald, and J. Shapiro.** 1977. Transposition of beta-lactamase locus from RP1 into *Pseudomonas putida* degradative plasmids. J. Bacteriol. **129**:809–814.
4. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the p15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
5. **Curtiss, R., III, M. Inoue, D. Pereia, J. C. Hsu, L. Alexander, and L. Rock.** 1977. Construction and use of safer bacterial host strains for recombinant DNA research, p. 99–114. *In* W. A. Scott and R. Werner (ed.), Molecular cloning of recombinant DNA. Academic Press, Inc., New York.
6. **Davis, R. W., M. Simon, and N. Davidson.** 1971. Electron microscope heteroduplex methods for mapping regions of base sequence homology in nucleic acids. Methods Enzymol. **21**:413–428.
7. **Grindley, N. D. F., J. N. Grindley, and E. S. Anderson.** 1972. R factor compatibility groups. Mol. Gen. Genet. **119**:287–297.
8. **Hedges, R. W., N. Datta, P. Kontomichalou, and J. T. Smith.** 1974. Molecular specificities of R factor-determined beta-lactamases: correlation with plasmid compatibility. J. Bacteriol. **117**:56–62.
9. **Hedges, R. W., and A. E. Jacob.** 1974. Transposition of ampicillin resistance from RP4 to other replicons. Mol. Gen. Genet. **132**:31–40.
10. **Hedges, R. W., and M. Matthew.** 1979. Acquisition by *Escherichia coli* of plasmid-borne β-lactamase normally confined to *Pseudomonas* spp. Plasmid **2**:269–278.
11. **Heffron, F., R. Sublett, R. W. Hedges, A. Jacob, and S. Falkow.** 1975. Origin of the TEM beta-lactamase gene found on plasmids. J. Bacteriol. **122**:250–256.
12. **Ike, Y., H. Hashimoto, K. Motohashi, and S. Mitsuhashi.** 1980. Isolation and characterization of a composite plasmid Rms201 mutant temperature sensitive for replication. J. Bacteriol. **141**:577–583.
13. **Katsu, K., M. Inoue, and S. Mitsuhashi.** 1981. Plasmid-mediated carbenicillin hydrolyzing beta-lactamases of *Proteus mirabilis*. J. Antibiot. **34**:1504–1506.
14. **Kopecko, D. J., J. Brevet, and S. N. Cohen.** 1976. Involvement of multiple translocating DNA sequence and recombinational hotspots in the structural evolution of bacterial plasmids. J. Mol. Biol. **108**:333–360.
15. **Kopecko, D. J., and S. N. Cohen.** 1975. Site-specific *recA*-independent recombination betweeen bacterial plasmids: involvement of palindromes at the recombinational loci. Proc. Natl. Acad. Sci. U.S.A. **72**:1373–1377.
16. **Kupersztoch-Portnoy, Y. M., M. A. Lovett, and D. R. Helinski.** 1974. Strand and site specificity of the relaxation event for the relaxation complex of the antibiotic resistance plasmid R6K. Biochemistry **13**:5484–5490.
17. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Matthew, M., and R. W. Hedges.** 1976. Analytical isoelectric focusing of R factor-determined β-lactamases: correlation with plasmid compatibility. J. Bacteriol. **125**:713–718.
20. **Mitsuhashi, S., and M. Inoue.** 1981. New β-lactam antibiotics: antibacterial activities and inducibility of β-lactamase formation, p. 361–375. *In* M. Salton and G. D. Shockman (ed.), β-Lactam antibiotics: mode of action, new developments and future prospects. Academic Press, Inc., New York.
21. **Uhlin, B. E., and K. Nordstrom.** 1977. R plasmid gene dosage effects in *Escherichia coli* K12: Copy mutants of the R plasmid R1*drd*-19. Plasmid **1**:1–7.
22. **Tanaka, N., J. H. Cramer, and R. H. Rownd.** 1976. *Eco*RI restriction endonuclease map of the composite R plasmid NR1. J. Bacteriol. **127**:619–636.
23. **Tanaka, T., Y. Nagai, H. Hashimoto, and S. Mitsuhashi.** 1969. Distribution of R factor among *Shigella* strains isolated in Japan. Jpn. J. Microbiol. **13**:187–191.
24. **Waley, S. G.** 1974. A spectrophotometric assay of β-lactamase action on penicillins. Biochem. J. **139**:780–789.
25. **Yamada, Y., K. L. Calame, J. N. Grindley, and D. Nakada.** 1979. Location of ampicillin resistance transposon, Tn*1701*, in a group of small, nontransferring plasmids. J. Bacteriol. **137**:990–999.
26. **Yamamoto, T., R. Katoh, A. Shimazu, and S. Yamagishi.** 1980. Gene expression of ampicillin resistance transposons, Tn*2601* and Tn*2602*. Microbiol. Immunol. **24**:479–494.
27. **Yamamoto, T., M. Tanaka, R. Baba, and S. Yamagishi.** 1981. Physical and functional mapping of Tn*2603*, a transposon encoding ampicillin, streptomycin, sulfonamide, and mercury resistance. Mol. Gen. Genet. **181**:464–469.
28. **Yamamoto, T., M. Tanaka, C. Nohara, Y. Fukunaga, and S. Yamagishi.** 1981. Transposition of the oxacillin-hydrolyzing penicillinase gene. J. Bacteriol. **145**:808–813.
29. **Yun, T., and D. Vapnek.** 1977. Structure and location of antibiotic resistance determinants in bacteriophage P1Cm and P7 (ϕAmp), p. 229–234. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.