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RSF1010 Plasmid as a Potentially Useful Vector in *Pseudomonas* Species

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RSF1010 plasmid DNA was introduced into *Pseudomonas putida* and *P. aeruginosa* cells and maintained stably, suggesting the potential usefulness of this plasmid as a vector in *Pseudomonas* species. The number of copies of RSF1010 was 43 per chromosome equivalent in *P. putida* cells.

Bacteria belonging to the genus *Pseudomonas* have a wide variety of habitats in soil, fresh water, and marine environments and on plants, and they have a nutritional versatility to use a variety of organic compounds as sole carbon source. Recently, several plasmids that confer the ability to degrade octane, camphor, salicylate, xylene, or naphthalene were isolated from *P. putida* strains (3). Chakrabarty et al. (5) reported the transformation of *P. putida* with RP1 plasmid DNA, which has a mass of 38 × 10^6 daltons (38 Mdal). Nagahari et al. (9) conjugally transferred the RP4-*trp* hybrid plasmid, which contained the tryptophan operon of *Escherichia coli* into a *P. aeruginosa trp* strain, causing the synthesis of a derepressed level of tryptophan synthetase in *P. aeruginosa* cells. The RP4 (identical to RP1) plasmid has a wide host range among gram-negative bacteria, but because of its high molecular weight, it is difficult to handle. Failure to construct a mini RP4 plasmid with various restriction endonucleases suggests a scattering of the genes that are essential to the replication and maintenance of RP4 plasmid (1). It is desirable to find an appropriate cloning vector in *Pseudomonas* species, but most of the plasmids found in *Pseudomonas* species have masses >20 Mdal. We found that RSF1010 (5.5 Mdal) (6), which has one site susceptible to *Eco*RI restriction endonuclease and has been used as a cloning vector in *E. coli* (10), can be introduced into *P. aeruginosa* and *P. putida* strains and maintained stably in these strains.

All chemicals and antibiotics used for bacterial cultures and DNA preparation were described previously (10). *E. coli* J5 carrying RSF1010, which confers resistance to streptomycin and sulfonamide, was described (10). *P. putida* ATCC 12633 and *P. aeruginosa* M12 (9) strains, requiring tryptophan for growth, were used as recipient cells. L broth consisting of 1 liter of water, 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, and 1 g of glucose was used for growing bacteria. The medium of Vogel and Bonner (12) was used as the minimal medium for *P. putida*. Nutrient broth was used in the transformation experiment of *P. aeruginosa*. Plasmid DNA was purified according to the method described previously (10). The amount of plasmid DNA was determined by CsCl-ethidium bromide density gradient centrifugation according to the method of Matsubara et al. (8) with a slight modification as follows: a 20-ml Vogel and Bonner medium culture of *P. putida* containing RSF1010 labeled with [^{3}H]adenosine (20 μCi/ml) was harvested, washed twice with half the original volume of 20 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 8.0)-0.14 M NaCl, and suspended in 1.5 ml of 40 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 8.5)-25 mM ethylenediaminetetraacetic acid. The cells were treated with lysozyme and ruptured with an equal volume of 0.75% Sarkosyl NL. The cleared lysate was incubated at 65°C for 8 min and then treated with RNase and Pronase. To the whole lysate, solid CsCl was added with the ethidium bromide solution to $\rho = 1.575$. After centrifugation at 38,000 rpm for 40 h at 15°C, the gradients were collected and assayed for radioactivity as described (10). The procedures of electron microscopy and agarose-gel electrophoresis were described previously (10).

P. putida ATCC 12633 cells were first transformed to a streptomycin-resistant phenotype (Sm^r) with RSF1010 plasmid DNA derived from *E. coli* strain J5. To compare the transformation efficiency with RSF1010 from different strains, RSF1010 plasmid DNA isolated from *E. coli* J5 and transformed *P. putida* ATCC 12633 cells were used. Transformation efficiency of *P. putida* ATCC 12633 with RSF1010 derived from the transformed cells was more than 100-fold

higher than that of *E. coli* (Table 1), yielding 2.8 $\times 10^5$ transformants per μg of DNA. This suggests the existence of a restriction system in *P. putida* ATCC 12633 strain. Table 1 also shows that RSF1010 plasmid DNA from *E. coli* can transform *P. aeruginosa* cells to the Sm^r phenotype. The time necessary for the expression of streptomycin resistance in antibiotic-free medium after transformation was found to be 8 h for *P. putida* and 4 h for *P. aeruginosa* cells (data not shown).

The mass of RSF1010 in three different strains was measured by electron microscopy by using ColE1 DNA (4.2 Mdal [2]) as internal standard. Table 2 shows that plasmid RSF1010 from three different strains were identical in mass (5.5 Mdal), suggesting that RSF1010 plasmid DNA was introduced into *P. putida* and *P. aeruginosa* cells in an intact form. Each RSF1010 plasmid DNA has one site susceptible to *Eco*RI and *Hpa* I, and three sites to *Hinc*II restriction endonucleases. The digestion patterns of RSF1010 plasmid DNAs obtained from *E. coli, P. putida,* and *P. aeruginosa* cells of these restriction endonucleases were identical (data not shown). The amount of plasmid DNA in *P. putida* ATCC 12633 measured by CsCl-ethidium bromide density gradient was 5.9% of its chromosomal DNA (data not shown). Assuming the molecular weight of *P. putida* chromosomal DNA to be the same as that of *P. oleovorans* (4×10^9 [7]), the number of RSF1010 plasmid DNA in the cell is 43 copies per chromosome.

TABLE 1. *Transformation efficiency for streptomycin-resistant phenotype specified by RSF1010 DNA*[a]

Recipient	RSF1010 from:	
	E. coli J5	*P. putida* ATCC 12633
P. putida ATCC 12633	1.8×10^{-7}[b]	2.7×10^{-5}
P. aeruginosa M12	6.7×10^{-7}	ND[c]

[a] Transformation of *P. putida* and *P. aeruginosa* by $CaCl_2$ treatment was carried out by the method of Chakrabarty et al. (5) and Sano and Kageyama (11), respectively.

[b] Transformation efficiencies are represented as the number of transformants per recipient cell.

[c] ND, Not done.

TABLE 2. *Mass of RSF1010 from different strains*

Strain	Mass (Mdal) ± SD[a]	No. of molecules measured
E. coli J5	5.5 ± 0.3	18
P. putida ATCC 12633	5.5 ± 0.2	12
P. aeruginosa M12	5.5 ± 0.3	16

[a] RSF1010 and ColE1 (used as internal standard) were converted to the linear form with *Eco*RI simultaneously, and the molecular weights of RSF1010 were measured relative to ColE1 DNA. There were 10 to 20 ColE1 molecules measured in each sample. SD, Standard deviation.

TABLE 3. *Stability of RSF1010 in different strains*

Host strain	Stability[a] (%)
E. coli J5	98.9
P. putida ATCC 12633	99.8
P. aeruginosa M12	92.6

[a] Stability is indicated as the percentage of cells that was resistant to streptomycin after one generation in an antibiotic-free L broth. The concentration of streptomycin in the test plates was 10 μg/ml for *E. coli* and 100 μg/ml for *P. putida* and *P. aeruginosa.*

TABLE 4. *MIC of streptomycin for three strains carrying RSF1010*

Strain	MIC[a]	
	− Plasmid	+ Plasmid
E. coli J5	5 μg/ml	50 μg/ml
P. putida ATCC 12633	10 μg/ml	10 mg/ml
P. aeruginosa M12	25 μg/ml	5 mg/ml

[a] Nutrient agar plates were used for determination of minimum inhibitory concentration (MIC).

Table 3 shows the percentage of cells that were resistant to streptomycin after they were cultured in an antibiotic-free medium. This indicates that the RSF1010 plasmid was more stable in *P. putida* cells than in *E. coli* or *P. aeruginosa* cells. Table 4 shows the minimal inhibitory concentration of streptomycin for three strains carrying the RSF1010 plasmid. Compared with *E. coli, P. putida* and *P. aeruginosa* were much more resistant to streptomycin.

This report suggests the potential usefulness of RSF1010 plasmid DNA as a vector in *Pseudomonas* species. RSF1010 plasmid, which confers resistance to streptomycin and sulfonamide, is nonconjugative and can be used as a vector by using *Eco*RI restriction endonuclease in *E. coli* (10). Chakrabarty described several advantages of *P. putida* over *E. coli* as a host bacterium (4). We recently constructed a hybrid plasmid in *P. putida* consisting of an RSF1010 and an *Eco*RI fragment of *P. putida* chromosomal DNA, which could transform the *P. putida* Leu^- phenotype to Leu^+ (manuscript in preparation). In *P. aeruginosa,* RSF1010 is compatible with RP4, which belongs to the P1 compatability group (unpublished data).

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LITERATURE CITED

1. **Barth, P. T., and N. J. Grinter.** 1977. Map of plasmid RP4 derived by insertion of transposon C. J. Mol. Biol. 113:455–474.

2. **Bazaral, M., and D. R. Helinski.** 1968. Circular DNA forms of colicinogenic factors E1, E2, and E3 from *Escherichia coli*. J. Mol. Biol. **36**:185–194.
3. **Chakrabarty, A. M.** 1976. Plasmids in *Pseudomonas*. Annu. Rev. Genet. **10**:7–30.
4. **Chakrabarty, A. M.** 1976. Molecular cloning in *Pseudomonas*, p. 579–582. *In* D. Schlessinger (ed.), Microbiology—1976. American Society for Microbiology, Washington, D.C.
5. **Chakrabarty, A. M., J. R. Mylroie, D. A. Friello, and J. G. Vacca.** 1975. Transformation of *Pseudomonas putida* and *Escherichia coli* with plasmid-linked drug-resistance factor DNA. Proc. Natl. Acad. Sci. U.S.A. **72**:3647–3651.
6. **Guerry, P., J. V. Embden, and S. Falkow.** 1974. Molecular nature of two nonconjugative plasmids carrying drug resistance genes. J. Bacteriol. **117**:619–630.
7. **Lethbak, A., C. Christiansen, and A. Stenderup.** 1970. Bacterial genome sizes determined by DNA renaturation studies. J. Gen. Microbiol. **64**:377–380.
8. **Matsubara, K., Y. Takagi, and T. Mukai.** 1975. In vitro construction of different oligomeric forms of λdv DNA and studies on their transforming activities. J. Virol. **16**:479–485.
9. **Nagahari, K., Y. Sano, and K. Sakaguchi.** 1977. Derepression of *E. coli trp* operon on interfamilial transfer. Nature (London) **266**:745–746.
10. **Nagahari, K., T. Tanaka, F. Hishinuma, M. Kuroda, and K. Sakaguchi.** 1977. Control of tryptophan synthetase amplified by varying the numbers of composite plasmids in *Escherichia coli* cells. Gene **1**:141–152.
11. **Sano, Y., and M. Kageyama.** 1977. Transformation of *Pseudomonas aeruginosa* by plasmid DNA. J. Gen. Appl. Microbiol. **23**:183–186.
12. **Vogel, H. J., and D. M. Bonner.** 1965. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.