TRANSDUCTION OF PLASMID DNA CONTAINING THE *ermE* GENE AND EXPRESSION OF ERYTHROMYCIN-RESISTANCE IN STREPTOMYCETES

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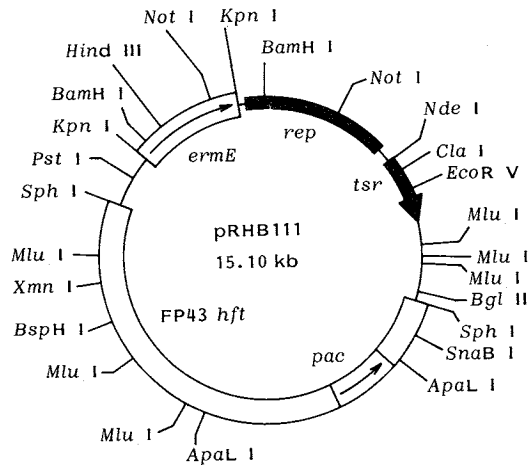
Plasmid pIJ702[1] is a useful vector for the cloning and expression of streptomycete and mammalian genes in *Streptomyces lividans*.[2~4] pIJ702 or its derivatives have broad host range, replicating in many *Streptomyces* species and in other actinomycetes (see ref 2~6 and refs therein). We have been interested in expanding the utility of pIJ702 by making it transducible. We cloned a segment of bacteriophage FP43 DNA (*hft*) into the *Sph* I site of pIJ702, and the resulting plasmid (pRHB101) was transducible by FP43 from *Streptomyces griseofuscus* ATCC 23916 into many streptomycetes.[6~8] The *hft* region mediating transduction contains an origin for headful packaging, or *pac* site.[9]

To use this transducible plasmid to genetically manipulate streptomycetes, we needed to know if DNA could be inserted into pRHB101 without disrupting transduction or plasmid stability. To test this, we inserted the *ermE* gene from *Saccharopolyspora erythraea*[10,11] into the *Kpn* I site of pRHB101 (Fig. 1) and introduced the resulting plasmid (pRHB111) into *S. griseofuscus* by protoplast transformation as described.[6] An FP43 lysate was prepared on *S. griseofuscus* (pRHB111)[6], and was used to transduce *S. griseofuscus*, *Streptomyces ambofaciens* ATCC 15154, *Streptomyces albus* P, *Streptomyces fradiae* M1 and *Streptomyces thermotolerans* NRRL15270. *S. griseofuscus* and *S. ambofaciens* were chosen as recipients since they do not restrict DNA prepared from other species.[6,12] *S. albus* P expresses *Sal*PI (*Pst*I)[13] and moderately restricts pRHB101, which contains one *Pst* I site.[6] *S. thermotolerans* and *S. fradiae* M1 restrict FP43 plaque formation, but permit transduction of pRHB101 at fairly high frequencies when cells are grown at 39°C to mid-exponential phase.[8] *S. ambofaciens*, *S. griseofuscus*, *S. fradiae* and *S. thermotolerans* also produce macrolide antibiotics,[12] and *S. ambofaciens*, *S. fradiae* and *S. thermotolerans* express macrolide antibiotic resistances that have been cloned.[14~19] Table 1 shows that pRHB111 was transduced into all five species at frequencies similar to those observed with the parent plasmid pRHB101, indicating that DNA can be inserted into the *Kpn* I site of pRHB101 without disrupting transduction. Plasmid DNA was isolated from transductants of all species, except *S. fradiae*, as described[3] and cleaved with *Bam*H I, *Hin*d III, *Kpn* I, *Nco* I and *Sph* I, and the restriction fragment patterns were observed after agarose gel electrophoresis.[20] The restriction fragment patterns were identical to those obtained from the original plasmid, indicating that pRHB111 did not undergo

Fig. 1. Construction of plasmid pRHB111.



A 1.6-kb *Kpn* I fragment containing the *ermE* gene[3] was isolated from plasmid pKC488.[11] The fragment was inserted into the *Kpn* I site of plasmid pRHB101 and introduced by transformation into *S. griseofuscus* as described.[6]

Table 1. Transduction of plasmid DNA in streptomyces.[a]

Strain	Transduction frequency by[b]	
	pRHB101	pRHB111
Streptomyces albus P	1×10^{-7}	2×10^{-7}
S. ambofaciens	3×10^{-6}	1×10^{-5}
S. griseofuscus	1×10^{-4}	2×10^{-4}
S. fradiae M1	1×10^{-5}	7×10^{-6}
S. thermotolerans	3×10^{-6}	7×10^{-6}

[a] Transductions by FP43 were carried out generally as described,[6] using modifications for *S. fradiae* M1[7] and for *S. ambofaciens* and *S. thermotolerans*[8] that optimize transduction frequencies.

[b] Transduction frequency is the number of transductants per PFU determined on *S. griseofuscus*.[6]

Table 2. Expression of erythromycin-resistance in transductants containing pRHB111.[a]

Strain	Erythromycin (μg/ml)	Relative EOP +pRHB111	Relative EOP −pRHB111
Streptomyces albus P	0	1.0	1.0
	100	—	$<3\times10^{-7}$
	400	0.7	$<3\times10^{-7}$
	1,000	0.6	$<3\times10^{-7}$
S. ambofaciens	0	1.0	1.0
	200	1.0	$<1\times10^{-8}$
	400	0.5	$<1\times10^{-8}$
	800	0.7	$<1\times10^{-8}$
	1,000	0.8	$<1\times10^{-8}$
S. griseofuscus	0	1.0	1.0
	200	1.6	8×10^{-7}
	500	0.9	$<3\times10^{-7}$
	1,000	0.6	$<3\times10^{-7}$
S. thermotolerans	0	1.0	1.0
	25	—	2×10^{-4}
	75	0.8	$<2\times10^{-7}$
	400	0.8	—

[a] Parental strains and transductants containing pRHB111 were grown in TS broth at 29°C (*S. albus* P, *S. ambofaciens* and *S. griseofuscus*) or 39°C (*S. thermotolerans*), then fragmented by ultrasound as described.[24] Mycelial fragments were plated on modified R2 agar plates[24] containing different concentrations of erythromycin, and colonies were counted at 5 days.

deletion or rearrangement at appreciable rates in the four species tested.

Transductants of four of the streptomycetes were analyzed for the expression of erythromycin-resistance by efficiency of plating at various erythromycin concentrations. Table 2 shows that mycelial fragments of transductants expressed levels of erythromycin-resistance that resulted in efficiencies of plating of over 50% at 400 to 1,000 μg/ml erythromycin. The strains lacking the plasmid were highly sensitive to erythromycin at these and lower erythromycin concentrations. These results indicated that plasmid was stably maintained and expressed erythromycin-resistance in the four species tested. *S. fradiae* M1 transductants were not tested for erythromycin-resistance since *S. fradiae* expresses erythromycin-resistance by constitutive monomethylation,[19] and erythromycin-inducible dimethylation of position 2058 of 23S rRNA.[15,17]

Both *S. ambofaciens* and *S. thermotolerans* encode macrolide-resistance genes.[14,16,18] The *S. thermotolerans carB* gene[14] encodes an enzyme that monomethylates position 2058 of 23S rRNA to confer high level resistance to carbomycin, but not to erythromycin.[18] A second carbomycin-resistance gene, *carA*, in *S. thermotolerans* apparently is not involved in dimethylation of position 2058.[14] Thus it appears that the expression of *ermE*, which encodes an enzyme that dimethylates position 2058 in 23S rRNA,[10,21] and which is driven by two promoters that express in streptomycetes,[22,23] may be generally useful as a selectable marker in streptomycetes, even in some species that express resistance to 16-membered macrolide antibiotics.

In summary, our results indicate that DNA can be inserted into the *Kpn* I site of pRHB101 without disrupting plasmid stability or transducibility, and the inserted DNA can be expressed in transductants. Thus pRHB101 and derivatives should be useful vectors for cloning and transfer of genes into many streptomycete species.

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