Communication to the Editor

TRANSDUCTION OF PLASMID DNA IN MACROLIDE PRODUCING STREPTOMYCETES

Sir:

We recently described a new method to transfer plasmid DNA between different species of *Streptomyces* by bacteriophage FP43 transduction[1]. Transduction was mediated by a 8-kb segment of bacteriophage FP43 DNA cloned into plasmid pIJ702: The resulting plasmid, pRHB101, was packaged in bacteriophage heads as linear concatemers and was transduced into about 80% of the *Streptomyces* species tested[1]. We showed that the efficiency of transduction can vary markedly depending on the cell growth conditions, and that under optimum growth conditions both highly restricting and relatively nonrestricting strains of *Streptomyces fradiae* can be transduced at high efficiencies[1~4]. Transduction frequencies varied by 1,000-fold with growth conditions in *S. fradiae*, and optimum transduction frequencies were obtained with cells grown to mid to late exponential phase at 39°C[3].

We were interested in exploring the effects of cell growth conditions on transduction in other streptomycetes that produce macrocyclic lactones, since these strains are potential recipients for cloned macrolide genes to produce hybrid structures with potentially useful activities[2,5~7].

We compared several macrolide-producing *Streptomyces* for their ability to support plaque formation by ten broad host range bacteriophages for *Streptomyces* species. It has been shown that inhibition of plaque formation by one or more of these phages correlates well with the expression of restriction[8~10]. Table 1 shows that of the seventeen macrolide producers tested, only *Streptomyces ambofaciens*, *Streptomyces griseofuscus* and *Streptomyces platensis* permit plaque formation by all ten bacteriophages. Therefore, it is likely that most or all of the other species express some restriction.

We grew two nonrestricting (*S. ambofaciens* and *S. griseofuscus*) and two apparently restricting species (*Streptomyces cirratus* and *Streptomyces thermotolerans*) at 29 and 39°C to different growth phases and determined transduction frequencies.

Table 1. Summary of bacteriophage plaque formation on macrolide-producing streptomycetes[a].

Species	Macrolide produced[b]	Plaque formation by bacteriophage[c]									
		FP4	FP22	FP43	FP46	FP50	FP55	FP60	FP61	VP11	R4
Streptomyces ambofaciens	Spiramycin	+	+	+	+	+	+	+	+	+	+
S. antibioticus	Oleandomycin	−	−	+/−	−	+/−	−	−	−	−	−
S. avermitilis	Avermectin	+	+	+	−	+	+	+	+	+	+
S. cirratus	Cirramycin	+	−	−	−	−	−	−	−	−	−
S. eurythermus	Angolamycin	+	−	−	−	−	−	−	−	−	−
S. felleus	Pikromycin	−	−	−	−	−	−	−	−	−	−
S. fradiae	Tylosin	+	+	−	−	+	−	+	+	+	−
S. fungicidicus	Espinomycin	−	+	−	−	−	−	−	−	+	−
S. griseofuscus	Lankacidin	+	+	+	+	+	+	+	+	+	+
S. halstedii	Carbomycin	+	+	−	+	+	+	+	+	+	+
S. hygroscopicus	Maridomycin	−	+	+/−	+	−	−	+	−	−	−
S. macrosporeus	Carbomycin	+	−	+	−	−	−	−	−	−	−
S. mycarofaciens	Espinomycin	+	+	−	+	+	+	−	+	+	+
S. narbonensis	Narbomycin	−	−	−	−	+	−	−	+	+	−
S. platensis	Platenomycin	+	+	+	+	+	+	+	+	+	+
S. thermotolerans	Carbomycin	+	+	−	+	+	−	−	+	+	+
S. venezuelae	10-Deoxymethymycin	−	−	−	−	−	−	−	−	−	−

[a] Cells were grown at 29°C to stationary phase for plaque assays[8]. Some of the data are from Cox and BALTZ[8].

[b] See ŌMURA[11], and Cox and BALTZ[8].

[c] +: Plaque formation in streak tests[8], −: no plaque formation, +/−: plaque formation observed with some but not all lysates.

Fig. 1. Effects of growth temperature and growth phase on transduction of macrolide-producing streptomycetes.

(A) *Streptomyces griseofuscus* grown at 29°C, (B) *S. ambofaciens* grown at 29°C (○, ●) or 39°C (□, ■), (C) *S. cirratus* grown at 29°C (○, ●) or 39°C (□, ■), (D) *S. thermotolerans* grown at 29°C (○, ●) or 39°C (□, ■). Transductants: Closed, absorbance: open.

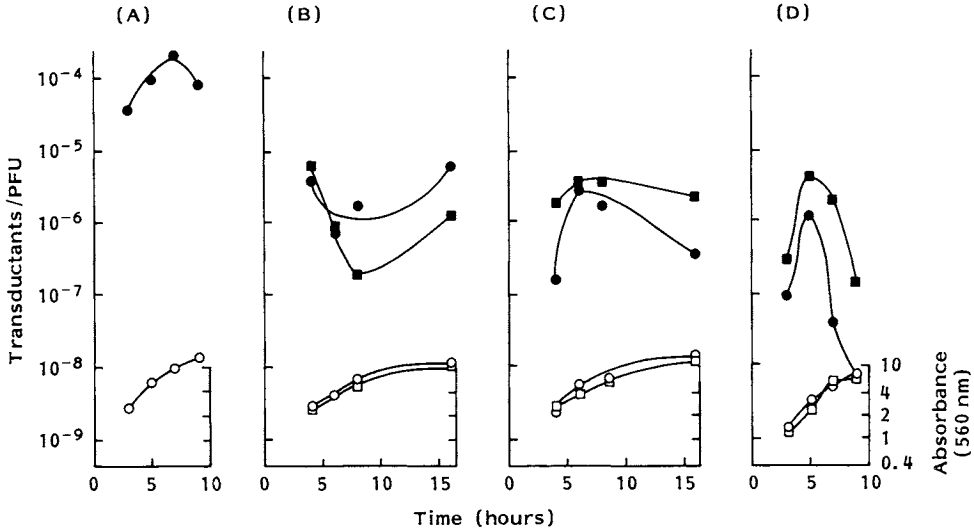

Table 2. Summary of FP43 transduction and plaque formation on macrolide-producing streptomycetes[a].

Strain	Plaque formation[b]	Transduction	Growth condition[c]	Transduction frequency[d]
Streptomyces ambofaciens	+	+	2	6×10^{-6}
S. antibioticus	+/−	−	1~4	$<3\times10^{-10}$
S. avermitilis	+	+	2[e]	5×10^{-7}
S. cirratus	−	+	3	4×10^{-6}
S. eurythermus	−	+	2[e]	4×10^{-9}
S. felleus	−	+	2[e]	6×10^{-6}
S. fradiae	−	+	3	1×10^{-6}
S. fungicidicus	−	−	1~4	$<3\times10^{-8}$
S. griseofuscus	+	+	1	2×10^{-4}
S. halstedii	−	−	2	$<1\times10^{-8}$
S. hygroscopicus	+/−	+	2	1×10^{-7}
S. macrosporeus	+	+	2[e]	1×10^{-6}
S. mycarofaciens	−	−	1~4	$<3\times10^{-8}$
S. narbonensis	−	+	4	1×10^{-9}
S. platensis	+	+	2[e]	1×10^{-5}
S. thermotolerans	−	+	3	4×10^{-6}
S. venezuelae	−	+	4	8×10^{-8}

[a] Includes some information from McHENNEY and BALTZ[1].
[b] From Table 1.
[c] 1: 29°C and exponential growth phase, 2: 29°C and stationary phase, 3: 39°C and exponential phase, 4: 39°C and stationary phase.
[d] Transduction frequency, transductants per PFU. The condition cited gave the highest transduction frequency unless stated otherwise.
[e] Only condition tested.

Transduction frequencies with *S. griseofuscus* cells grown at 29°C varied by only 5-fold with cell growth phase (Fig. 1), and the number of transductants paralleled the optical density of the culture except with cells entering stationary phase. The efficiency of transduction dropped with *S. ambofaciens* cells

in late exponential growth phase, then increased with cells in stationary phase (Fig. 1); higher frequencies of transformants were obtained with cells grown at 29°C rather than at 39°C. With *S. cirratus* and *S. thermotolerans*, strains that restrict FP43 plaque formation (Table 1), the highest transduction frequencies were obtained from cells grown at 39°C to late exponential growth phase (Fig. 1). The transduction frequencies in *S. thermotolerans* declined 30- to 100-fold with cells entering stationary phase, whereas transduction frequencies declined less dramatically with *S. cirratus* cells entering stationary phase. The maximum transduction frequencies observed with *S. thermotolerans*, *S. cirratus* and *S. ambofaciens* were similar ($\sim 10^{-5}$ per plaque forming unit (PFU)), but were about 10-fold lower than the maximum frequency obtained with *S. griseofuscus*.

Several other macrolide-producing streptomycetes were grown at 29 and 39°C to mid exponential or stationary phases, and different patterns of transduction were observed (Table 2). With *Streptomyces venezuelae* and *Streptomyces narbonensis*, the highest transduction frequencies were obtained with cells grown at 39°C to stationary phase; whereas with *Streptomyces hygroscopicus*, the highest transduction frequency was obtained with cells grown at 29°C to stationary phase. Only *Streptomyces antibioticus*, *Streptomyces fungicidicus*, *Streptomyces halstedii* and *Streptomyces mycarofaciens* were not transducible under the conditions tested, whereas ten of the seventeen species did not support FP43 plaque formation. Only *Streptomyces chryseus* (data not shown) could not be tested for transduction since it was resistant to thiostrepton.

The results presented here and elsewhere[3,4] indicate that restriction barriers in many streptomycetes, including macrolide producers, can be reduced by manipulating growth conditions before transduction. Under optimum conditions, greater than 75% of the strains tested were transducible by plasmid pRHB101. This suggests that bacteriophage FP43-mediated transduction of plasmid DNA may be a method to transfer antibiotic and other secondary metabolic genes into many macrolide-producing strains, including ones that are highly restricting for plasmid transformation or ones that are not readily transformed by existing procedures.

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(Received April 1, 1989)

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