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Transduction and Transformation of Plasmid DNA in *Streptomyces fradiae* Strains That Express Different Levels of Restriction

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We constructed nonrestricting strains of *Streptomyces fradiae* blocked in different steps in tylosin biosynthesis. Plasmid transformation frequencies were 10^3- to 10^4-fold higher and bacteriophage plating efficiencies were 10^4- to 10^8-fold higher in the nonrestricting strains than in the restricting strains. The efficiencies of transduction of plasmid pRHB101 in *S. fradiae* strains varied by over 1,000-fold, depending on growth conditions, and optimum transduction frequencies were obtained when cells were grown to mid-exponential phase at 39°C. Under these conditions, restricting and nonrestricting strains were transduced at frequencies that differed by only two- to fivefold.

We recently described a method to transduce plasmid DNA in many species of the genus *Streptomyces* (19). We cloned in plasmid pIJ702 a segment of bacteriophage FP43 DNA, designated *hft*, that mediated high-frequency transduction of the resulting plasmid (pRHB101) in *Streptomyces griseofuscus*. FP43 lysates prepared on *S. griseofuscus*(pRHB101) transduced about 80% of the *Streptomyces* species tested, including two species that produce restriction endonucleases that cut pRHB101 DNA, and many other species suspected of producing restriction enzymes. Transducing lysates contained pRHB101 packaged in phage heads as linear concatemers, which suggested that the *hft* segment may contain an origin for rolling-circle replication, a packaging initiation site, or both.

During our initial studies, we observed that transduction frequencies in two species increased if cells were grown at elevated temperature before transduction. One of the strains, *Streptomyces phaeochromogenes*, produces a restriction enzyme (*Sph*I) that cuts pRHB101 twice, which suggests that growth at elevated temperature might inhibit the expression or activity of *Sph*I.

We were interested in using transduction in *Streptomyces fradiae*, the producer of tylosin (5). Many mutants blocked in tylosin biosynthesis have been isolated and characterized (4, 10), and most of the *tyl* mutations have been complemented by gene cloning (5, 10, 12). A major obstacle for cloning in *S. fradiae* is restriction, since wild-type *S. fradiae* produces several restriction systems that can inhibit transformation (18).

In this report, we show that restricting and relatively nonrestricting derivatives of *S. fradiae* grown to optimal cell densities at elevated temperature are transduced by plasmid DNA at high efficiencies, whereas the restricting strains are transformed by plasmid DNA at frequencies 10^3- to 10^4-fold lower than are nonrestricting strains.

MATERIALS AND METHODS

Bacteria, bacteriophage, and plasmid. The streptomycete strains used are listed in Table 1. FP4, FP22, FP43, FP46, FP50, FP55, FP60, FP61, VP11, and R4 are broad-host-range bacteriophages of *Streptomyces* spp. (7–9, 11). Plasmids pIJ702 (14) and pHJL281 (16) were isolated as described previously (15, 17). pRHB101 is a plasmid derived from pIJ702 containing an insert of FP43 DNA that mediates high-frequency transduction of plasmid in streptomycetes (19). pKC434 is an ~51-kilobase cosmid containing *Streptomyces felleus* DNA (18). *Escherichia coli* DH5α was used to prepare the bifunctional plasmid pHJL281.

Media and culture conditions. Mycelia were grown in TS broth and fragmented into single cells for plaque assays by ultrasound as described previously (1). Cell growth was measured by A_{550} of fragmented mycelia (1). Growth on nitrate as the sole nitrogen source was determined on CD agar as described elsewhere (20). Expression of resistance to 50 μg of spectinomycin or 400 μg of tylosin per ml was determined as described previously (20). Bacteriophage plate stocks were prepared on NC agar, and plaque formation was determined as described previously (9).

Materials. Anti-FP43 antiserum was prepared as described elsewhere (19).

Mutagenesis. Mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was carried out as described previously (3, 4). Mutants defective in restriction were selected as described previously (18).

Conjugation. Conjugal matings were carried out as described previously (20).

Transformation. Transformation of host bacteria plasmid DNA was carried out as described previously (17).

Transduction. Transduction of plasmid pRHB101 to yield thiostrepton-resistant colonies was determined as described elsewhere (19). The standard transduction condition was to grow cells at 29°C to stationary phase, to sonicate mycelia, and to mix 100 μl of cells with 100 μl of appropriately diluted transducing lysate in an R2 soft agar overlay. The overlay was added to an NC agar plate and incubated at 34°C. Anti-FP43 antiserum was not generally used but was added in a nutrient soft agar overlay (19) for the experiment described in Fig. 1. Thiostrepton (final concentration, 25 μg/ml) was added in a nutrient soft agar overlay at 6 h as described previously (19). Antibiotic fermentations were carried out and analyzed by thin-layer chromatography for tylosin-related macrolides as described elsewhere (4).

RESULTS

Construction of nonrestricting *S. fradiae* strains blocked in specific steps in tylosin biosynthesis. We mutagenized *S. fradiae* PM41, a derivative of JS85 defective in several restriction systems (18), with *N*-methyl-*N'*-nitro-*N*-nitroso-

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TABLE 1. *Streptomyces* strains used

Strain	Relevant phenotype or property[a]	Derivation	Reference
S. fradiae			
M1	Tyl^r Tyl^+ Nar^- Spc^s Spo^-; restricting	Spontaneous mutant from C4	2, 17
JS85	Tyl^s Tyl^- Nar^+ Spc^r; restricting; deleted for many *tyl* genes	Spontaneous Spc^r mutant from JS82	5, 6, 20
GS50	Tyl^r $TylB^-$ Nar^- Spc^s; restricting	MNNG[b] mutagenesis of C4	4, 18; this report
GS48	Tyl^r $TylD^-$ Nar^- Spc^s; restricting	MNNG mutagenesis of C4	4; this report
GS16	Tyl^r $TylE^-$ Nar^- Spc^s; restricting	MNNG mutagenesis of C4	4; this report
GS15	Tyl^r $TylF^-$ Nar^- Spc^s; restricting	MNNG mutagenesis of C4	4, 18; this report
GS76	Tyl^r $TylH^-$ $TylD^-$ Nar^- Spc^s; restricting	MNNG mutagenesis of GS48	4; this report
GS88	Tyl^r $TylJ^-$ Nar^- Spc^s; restricting	MNNG mutagenesis of C4	10; this report
PM76	Tyl^s Tyl^- Nar^+ Spc^r; nonrestricting	MNNG mutagenesis of PM41	This report
PM73	Tyl^r $TylB^-$ Nar^+ Spc^r; nonrestricting	Conjugation between GS50 and PM41 followed by MNNG mutagenesis	18
PM78	Tyl^r $TylD^-$ Nar^+ Spc^r; nonrestricting	Conjugation between GS48 and PM76	This report
PM79	Tyl^r $TylE^-$ Nar^+ Spc^r; nonrestricting	Conjugation between GS16 and PM76	This report
PM77	Tyl^r $TylF^-$ Nar^+ Spc^r; nonrestricting	Conjugation between GS15 and PM76	This report
PM80	Tyl^r $TylH^-$ $TylD^-$ Nar^+ Spc^r; nonrestricting	Conjugation between GS76 and PM76	This report
PM82	Tyl^r $TylJ^-$ Nar^+ Spc^r; nonrestricting	Conjugation between GS88 and PM76	This report
S. griseofuscus	Nonrestricting host for phage plaque formation	ATCC 23916	9, 18
S. lividans TK23	Spc^r	NRRL 15826	13

[a] Tyl^r, Resistant tylosin at 400 μg/ml; Tyl^+, produces tylosin; Tyl^-, defective in tylosin production; $TylA^-$, $TylB^-$, $TylC^-$, etc., defective in specific steps in tylosin biosynthesis (4, 10; Table 3); Nar^+, grows on nitrate as the nitrogen source; Spc^r, resistant to spectinomycin at 50 μg/ml; Spo^+, proficient in sporulation; restricting, expresses several restriction systems; nonrestricting, defective in most or all restriction.

[b] MNNG, *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine.

guanidine and selected strains exhibiting increased transformability by plasmid pKC434. Several mutants were tested for plaque formation by bacteriophages that are restricted in wild-type *S. fradiae* strains; *S. fradiae* PM76 plated FP43, FP55, and FP62 at high efficiencies (Table 2) and was transformed by unmodified plasmid pKC434 at a frequency of 1.3×10^4 transformants per μg of DNA, whereas PM41 gave 3.2×10^1 transformants per μg of DNA. PM76 was also transformed by plasmids pFJ105 and pIJ702, which were modified for *S. fradiae* restriction by passage through *S. fradiae* M1, at frequencies of $>10^6$/μg of DNA and by plasmids pFJ105, pIJ702, and pHJL192, unmodified for *S. fradiae* restriction (i.e., prepared from *Streptomyces lividans* or *E. coli*), at frequencies of $\sim 10^5$, 10^5, and 10^6/μg of DNA, respectively. PM76 was crossed by conjugation with several mutants of *S. fradiae* blocked in tylosin biosynthesis,

TABLE 2. Efficiency of plating of bacteriophages on *S. fradiae* mutants and recombinants

Strain	Efficiency of plating of[a]:		
	FP43	FP55	FP62
JS85	$<2 \times 10^{-9}$	$<2 \times 10^{-8}$	$<1 \times 10^{-9}$
GS50 (*tylB*)	$<6 \times 10^{-9}$	$<2 \times 10^{-8}$	5×10^{-7}
GS48 (*tylD*)	$<6 \times 10^{-9}$	$<2 \times 10^{-8}$	5×10^{-7}
GS16 (*tylE*)	$<6 \times 10^{-9}$	$<2 \times 10^{-8}$	6×10^{-8}
GS15 (*tylF*)	$<3 \times 10^{-9}$	$<2 \times 10^{-8}$	5×10^{-8}
GS76 (*tylH tylD*)	$<6 \times 10^{-9}$	$<2 \times 10^{-8}$	$<1 \times 10^{-8}$
GS88 (*tylJ*)	4×10^{-6}	$<2 \times 10^{-8}$	8×10^{-7}
PM76	7×10^{-1}	3×10^{-1}	5×10^{-3}
PM78 (*tylD*)	5×10^{-2}	1×10^{-3}	3×10^{-4}
PM79 (*tylE*)	1×10^{-1}	7×10^{-3}	6×10^{-4}
PM77 (*tylF*)	3×10^{-1}	8×10^{-2}	2×10^{-3}
PM80 (*tylH tylD*)	1×10^{-1}	4×10^{-2}	7×10^{-3}
PM82 (*tylJ*)	4×10^{-2}	5×10^{-2}	1×10^{-3}

[a] Value for *S. griseofuscus* with each phage was 1.

and Tyl^r Spc^r recombinants were obtained. Recombinants were obtained at frequencies ranging from 4×10^{-5} to 4×10^{-2}. Typical recombinants were tested for growth on nitrate as the sole nitrogen source, and all expressed the Nar^+ phenotype of the recipient strain PM76. Recombinant strains that produced the macrolide products of the highly restricting parental strains were retained for further analysis.

Analysis of restriction in *S. fradiae* strains. The expression of restriction in the donor and recombinant strains were analyzed by bacteriophage efficiency-of-plating tests. Table 2 shows typical results obtained with donor and recombinant strains. The *tyl* mutants derived from a high-tylosin-producing strain (with GS designations) generally displayed plaque formation by bacteriophages FP43, FP55, and FP62 at very low to undetectable frequencies ($<10^{-9}$ to 10^{-6}). The *tylA*, *tylG*, and *tylK* mutants and the *tylI* and *tylD* double mutants also showed this pattern (not shown). The recombinant strains (with PM designations) showed plaque formation by the three phages at efficiencies that ranged from about 10^{-4} to 10^{-1}, similar to results for the relatively nonrestricting recipient strain PM76 (Table 2). Several other pairs of donor and recombinant strains expressing the same *tyl* mutant genes (e.g., *tylA*, *tylG*, and *tylK* mutants and *tylI tylD* double mutants) gave the same patterns of bacteriophage plating efficiencies (not shown). The strains listed in Table 2 were also analyzed for transformability by plasmids prepared from either *E. coli*(pHJL281) or *S. lividans* (pIJ702). GS50, GS48, GS16, GS15, GS76, GS88, and JS85 were inefficiently or not detectably transformed by plasmids pHJL281 and pIJ702 (Table 3), whereas PM76 and the recombinant strains (PM73, PM78, PM79, PM77, PM80, and PM82) were transformed at frequencies averaging $>10^4$/μg of DNA for both plasmids.

Time course of phage attachment and expression of thiostrepton resistance during transduction in *S. fradiae*. Before analyzing the effects of restriction on transduction in *S.*

TABLE 3. Transformation and transduction of *S. fradiae* mutants and recombinants by unmodified plasmids

Strain	Transformation frequency[a]		Transduction frequency[b]
	pHJL281	pIJ702	
JS85	$<2 \times 10^0$	$<4 \times 10^1$	5×10^4
GS50 (*tylB*)	1×10^1	3×10^2	
GS48 (*tylD*)	$<2 \times 10^0$	2×10^1	
GS16 (*tylE*)	6×10^0	1×10^2	9×10^2
GS15 (*tylF*)	$<2 \times 10^0$	3×10^2	3×10^4
GS76 (*tylH tylD*)	2×10^0	6×10^1	
GS88 (*tylJ*)	$<2 \times 10^0$	1×10^2	7×10^3
PM76	8×10^3	9×10^4	1×10^5
PM73 (*tylB*)	3×10^4	9×10^4	
PM78 (*tylD*)	2×10^4	8×10^3	
PM79 (*tylE*)	5×10^4	5×10^4	4×10^3
PM77 (*tylF*)	1×10^4	7×10^4	1×10^5
PM80 (*tylH tylD*)	4×10^3	3×10^4	
PM82 (*tylJ*)	2×10^4	2×10^4	2×10^4

[a] Transformants per microgram of plasmid DNA. pHJL281 was prepared from *E. coli*, and pIJ702 was prepared from *S. lividans*.

[b] Transductants per 10^{11} PFU, determined with pRHB101. Transductions were carried out with cells grown at 39°C to an A_{560} of 1 to 2. PFU were determined on *S. griseofuscus*, and transducing lysates were prepared on *S. griseofuscus*.

fradiae, we established the optimum times for addition of anti-FP43 antiserum and thiostrepton (Fig. 1). Addition of antiserum at time zero apparently caused the blockage of most phage attachment and resulted in a 100-fold reduction in the transduction frequency. The number of transductants insensitive to antiserum blockage increased exponentially for the first 2 h and then reached a half-maximal frequency by about 4 h. The first thiostrepton-resistant transductants were detectable at 1 h, after which time the transductant frequency increased exponentially to nearly 4 h. Maximum transductant frequencies were obtained by 6 to 8 h.

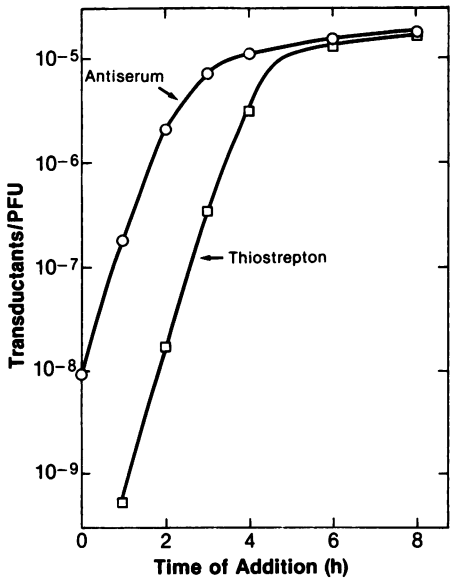

FIG. 1. Time course of phage attachment and expression of thiostrepton resistance during transduction. *S. fradiae* M1 was grown at 39°C to mid-exponential growth phase and then transduced in two ways: (i) anti-FP43 antiserum was added at different times (○) and thiostrepton was added at 8 h and (ii) thiostrepton was added at different times (□) and no antiserum was added.

Effects of cell growth phase and temperature on transduction in *S. fradiae* strains. We explored the effects of cell growth phase and growth temperature on transduction of restricting and relatively nonrestricting strains of *S. fradiae*. The relatively nonrestricting *S. fradiae* PM76 gave optimal transduction frequencies (about 10^5 transductants per 10^{11} PFU) when cells were grown at 39°C to an A_{560} of 1 to 2 (Fig. 2B). Transduction frequencies were 100-fold lower with cells taken at early stationary phase (A_{560} of 10) and were generally lower at comparable A_{560} values with cells grown at 29°C. *S. fradiae* M1, a restricting strain, gave optimal transduction frequencies (about $10^6/10^{11}$ PFU) when cells were grown at 39°C to an A_{560} of 1 to 2 (Fig. 2A).

We determined the effects of cell growth phase on transduction in two other highly restricting strains, GS15 and JS85, and in one relatively nonrestricting recombinant strain, PM77. JS85 and GS15 gave maximum frequencies of about 3×10^4 transductants per 10^{11} PFU (Fig. 2B and C), whereas PM77 gave a maximum frequency of about 1.5×10^5 transductants per 10^{11} PFU. The highest transduction frequencies were obtained with all of the strains when cells were grown at 39°C to mid- or late exponential phase; transduction frequencies declined about 100-fold with cells entering stationary growth phase. Two other pairs of restricting and relatively nonrestricting strains with identical blocks in tylosin biosynthesis were also grown under optimal conditions for transduction; the restricting strains gave transduction frequencies only two- to five-fold-lower than those for the nonrestricting strains (Table 3).

DISCUSSION

We recently demonstrated transduction of plasmid DNA in streptomycetes mediated by a segment of cloned bacteriophage FP43 DNA (*hft*) that caused the plasmid DNA to be packaged into FP43 phage heads as linear concatemers (19). We were interested in determining whether transduction could be used to circumvent restriction barriers in *S. fradiae*, a tylosin producer. Many strains of *S. fradiae* are highly restricting for plaque formation by certain bacteriophages and for transformation by plasmids prepared from cells other than *S. fradiae* (9, 17, 18). It has been suggested that wild-type *S. fradiae* may express five or more restriction systems (18). We constructed a series of relatively nonrestricting *S. fradiae* strains blocked in specific steps in tylosin biosynthesis and analyzed them, along with a series of *tyl* mutants derived from a restricting strain, for efficiencies of bacteriophage plaque formation, protoplast transformation, and transduction of plasmid DNA. PM76 is deleted for most or all tylosin biosynthesis genes (5, 18) and was defective in several restriction systems, as judged by bacteriophage plating efficiencies and plasmid transformation efficiencies. We mated PM76 by conjugation with several highly restricting strains of *S. fradiae* blocked in different steps in tylosin biosynthesis and showed that the recombinant strains expressed chromosomal genes (*spc* and *nar*) of PM76 and tylosin genes of the restricting donor strains. As judged by bacteriophage plaque formation and transformation with plasmid DNA unmodified for *S. fradiae* restriction, the recombinant strains were as defective in restriction as was PM76. These results strongly suggest that the tylosin biosynthesis genes included in the analysis are transmissible by conjugation independent of any significant restriction genes. These results extend previous findings that *tylA*, *tylB*, *tylC*, *tylF*, *tylD*, and *tylI* are transmissible independent of certain

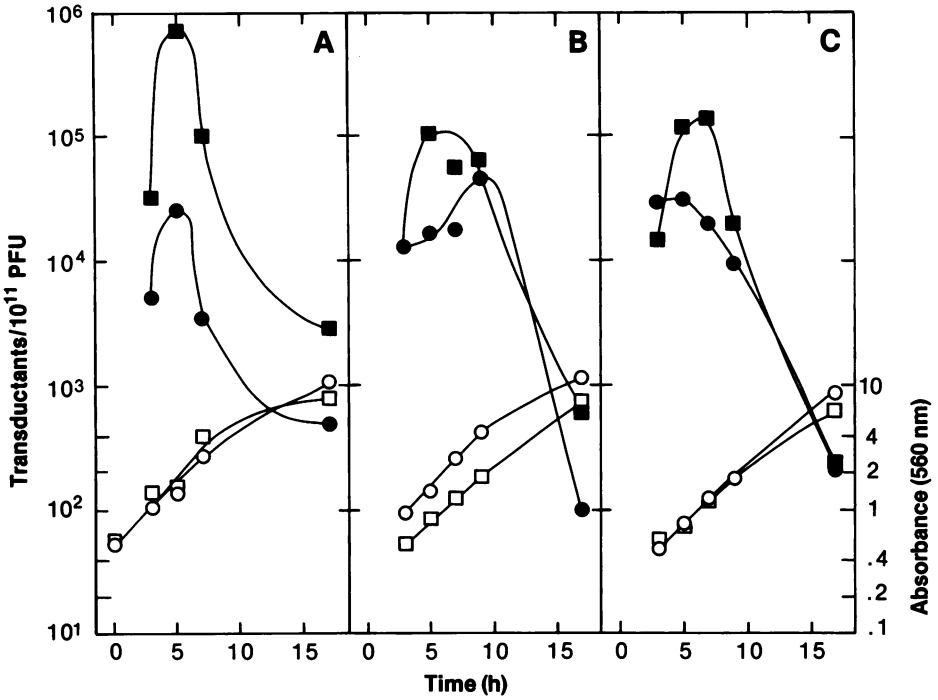

FIG. 2. Transduction of restricting and relatively nonrestricting *S. fradiae* strains as a function of cell growth phase and temperature. (A) *S. fradiae* M1 grown in TS broth at 29 (○, ●) or 39°C (□, ■); (B) *S. fradiae* PM76 (□, ■) and *S. fradiae* JS85 (○, ●) grown at 39°C; (C) *S. fradiae* PM77 (□, ■) and *S. fradiae* GS15 (○, ●) grown at 39°C. Solid symbols show the numbers of transductants per 10^{11} PFU, and open symbols show absorbance at 560 nm.

chromosomal genes (20) and that *tylB* and *tylF* genes can be transferred by conjugation independent of restriction genes (18). Recent cloning experiments also indicate that many *tyl* genes are physically linked (5, 10, 12).

We have shown here and elsewhere (18) that protoplasts of restricting strains of *S. fradiae* are transformed by plasmid DNA unmodified by passage through *S. fradiae* at frequencies 10^2- to 10^5-fold lower than are protoplasts of relatively nonrestricting mutants or recombinants. Also bacteriophages FP43, FP55, and FP62 did not form plaques or formed plaques at very low efficiencies on the restricting strains (i.e., 10^5- to 10^8-fold lower than value for the relatively nonrestricting strains). In an attempt to circumvent restriction in the highly restricting *S. fradiae* strains, we assessed the effects of cell growth phase and growth temperature on transduction with several *S. fradiae* strains. We noted that cells grown at 39°C were transduced at higher efficiencies than were cells grown at 29°C; restricting and nonrestricting strains were efficiently transduced at only marginally different frequencies when cells were grown to mid- or late exponential growth phase at 39°C before transduction; a sporulation-defective mutant (M1) that expresses restriction gave the highest transduction frequencies; and restricting and nonrestricting strains showed greatly reduced transduction when cells were grown to stationary phase. The strong dependency of transduction efficiency on both growth phase and growth temperature in highly restricting strains might be correlated with the expression of restriction endonucleases. However, this explanation does not explain the substantial decreases in transduction frequencies also observed in nonrestricting strains in stationary growth phase. This drop in transduction frequency may be due to poor phage attachment, to lower recombination rates to convert linear concatemers to circular monomers, to a lower efficiency of establishment of stable plasmid replication, to slower expression of thiostrepton resistance, or to other factors.

The time course studies on phage attachment and expression of thiostrepton resistance indicated that after attachment of phage to mid-exponential-phase cells grown at 39°C, it takes about 2 h for the cells to become fully resistant to thiostrepton. Attachment was essentially complete by 4 h, and expression of thiostrepton resistance was complete by 6 h. It is not known whether the formation of circular monomers from linear concatemers and plasmid replication must occur to obtain efficient expression of the *tsr* gene. If recombination and plasmid replication must precede efficient *tsr* gene expression, then a slower rate of either process could account for at least part of the reduced frequency of transduction observed with stationary-phase cells.

The relatively effective circumvention of restriction by FP43-mediated transduction under certain conditions may be due in part to the concatemeric nature of the transducing DNA. Multiple tandem copies of plasmid may be less susceptible to restriction than are circular monomers, since tandem copies of plasmid present multiple opportunities for homologous recombination to construct complete monomeric plasmids.

The circumvention of restriction barriers in *S. fradiae* by plasmid transduction may allow for efficient transfer of cloned genes into any *S. fradiae* strain. This putative property might be useful for transferring cloned antibiotic biosynthesis genes into highly restricting but high-antibiotic-producing mutants of *S. fradiae* blocked in tylosin biosynthesis.

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LITERATURE CITED

1. **Baltz, R. H.** 1978. Genetic recombination in *Streptomyces fradiae* by protoplast fusion and cell regeneration. J. Gen. Microbiol. **107**:93–102.
2. **Baltz, R. H.** 1986. Mutation in *Streptomyces*, p. 61–94. *In* S. W. Queener and L. E. Day (ed.), The bacteria, vol. 9. Antibiotic-producing *Streptomyces*. Academic Press, Inc., New York.
3. **Baltz, R. H.** 1986. Mutagenesis in *Streptomyces* spp., p. 184–190. *In* A. L. Demain and N. A. Solomon (ed.), Manual of industrial microbiology and biotechnology. American Society for Microbiology, Washington, D.C.
4. **Baltz, R. H., and E. T. Seno.** 1981. Properties of *Streptomyces fradiae* mutants blocked in biosynthesis of the macrolide antibiotic tylosin. Antimicrob. Agents Chemother. **20**:214–225.
5. **Baltz, R. H., and E. T. Seno.** 1988. Genetics of *Streptomyces fradiae* and tylosin biosynthesis. Annu. Rev. Microbiol. **42**: 547–574.
6. **Baltz, R. H., and J. Stonesifer.** 1985. Phenotypic changes associated with loss of expression of tylosin biosynthesis and resistance genes in *Streptomyces fradiae*. J. Antibiot. **38**:1226–1236.
7. **Chater, K. F.** 1986. *Streptomyces* phages and their applications to *Streptomyces* genetics, p. 119–158. *In* S. W. Queener and L. E. Day (ed.), The bacteria, vol. 9. Antibiotic-producing *Streptomyces*. Academic Press, Inc., New York.
8. **Chater, K. F., and A. T. Carter.** 1979. A new, wide host-range, temperate bacteriophage (R4) of *Streptomyces* and its interaction with some restriction-modification systems. J. Gen. Microbiol. **115**:431–442.
9. **Cox, K. L., and R. H. Baltz.** 1984. Restriction of bacteriophage plaque formation in *Streptomyces* spp. J. Bacteriol. **159**:499–504.
10. **Cox, K. L., S. E. Fishman, J. L. Larson, R. Stanzak, P. A. Reynolds, W.-K. Yeh, R. M. Van Frank, V. A. Birmingham, C. L. Hershberger, and E. T. Seno.** 1986. The use of recombinant DNA techniques to study tylosin biosynthesis and resistance in *Streptomyces fradiae*. J. Nat. Prod. **49**:971–980.
11. **Dowding, J. E.** 1973. Characterization of a bacteriophage virulent for *Streptomyces coelicolor* A3(2). J. Gen. Microbiol. **76**:163–176.
12. **Fishman, S. E., K. Cox, J. L. Larson, P. A. Reynolds, E. T. Seno, W.-K. Yeh, R. Van Frank, and C. L. Hershberger.** 1987. Cloning genes for the biosynthesis of a macrolide antibiotic. Proc. Natl. Acad. Sci. USA **84**:8248–8252.
13. **Hopwood, D. A., G. Hintermann, T. Kieser, and H. M. Wright.** 1984. Integrated DNA sequences in three streptomycetes from related autonomous plasmids after transfer to *Streptomyces lividans*. Plasmid **11**:1–16.
14. **Katz, E., C. J. Thompson, and D. A. Hopwood.** 1982. Cloning and expression of the tyrosinase gene from *Streptomyces antibioticus* in *Streptomyces lividans*. J. Gen. Microbiol. **192**: 2703–2714.
15. **Kieser, T.** 1984. Factors affecting the isolation of ccc DNA from *Streptomyces lividans* and *Escherichia coli*. Plasmid **12**:19–36.
16. **Larson, J. L., and C. L. Hershberger.** 1986. The minimal replicon of a streptomycete plasmid produces an ultrahigh level of plasmid DNA. Plasmid **15**:199–209.
17. **Matsushima, P., and R. H. Baltz.** 1985. Efficient plasmid transformation of *Streptomyces ambofaciens* and *Streptomyces fradiae* protoplasts. J. Bacteriol. **163**:180–185.
18. **Matsushima, P., K. L. Cox, and R. H. Baltz.** 1987. Highly transformable mutants of *Streptomyces fradiae* defective in several restriction systems. Mol. Gen. Genet. **206**:393–400.
19. **McHenney, M. A., and R. H. Baltz.** 1988. Transduction of plasmid DNA in *Streptomyces* spp. and related genera by bacteriophage FP43. J. Bacteriol. **170**:2276–2282.
20. **Stonesifer, J., P. Matsushima, and R. H. Baltz.** 1986. High frequency conjugal transfer of tylosin genes and amplifiable DNA in *Streptomyces fradiae*. Mol. Gen. Genet. **202**:348–355.