**Communication to the Editor**

# GENETIC TRANSFORMATION OF *MICROMONOSPORA ROSARIA* BY THE *STREPTOMYCES* PLASMID pIJ702

Sir:

Genetic transformation of protoplasts by plasmids derived from streptomycetes has been demonstrated in a number of *Streptomyces* species[1~7]. This has led to the cloning and expression of many streptomycete genes, including many genes involved in antibiotic biosynthesis[8~10]. Recently, protoplasts from three other genera of actinomycetes, *Amycolatopsis*[11], *Saccharopolyspora*[12] and *Thermomonospora*[13] have been transformed by vectors derived from streptomycete plasmids. Further, plasmid pIJ702 containing a cloned segment of bacteriophage FP43 DNA has been transduced by FP43 into species of *Chainia*, *Saccharopolyspora* and *Streptoverticillium*, as well as into many species of *Streptomyces*[14]. These studies indicated that broad host range streptomycete cloning vectors could be used to clone genes into several actinomycete genera.

We were interested in attempting to transform *Micromonospora rosaria* for two reasons. First, we wanted to further explore the host range of streptomycete plasmids, particularly in genera not closely related to streptomycetes[15]. In this regard, transduction of *M. rosaria* with phage FP43 was not demonstrated; whereas *Streptoverticillium* and *Chainia*, which are genera closely related taxonomically to *Streptomyces*, were transducible[14]. Secondly, we were interested in attempting to introduce cloned macrolide antibiotic biosynthetic genes into *M. rosaria* to produce hybrid macrolide antibiotics[16,17] containing the rosaramicin lactone structure[18]. To accomplish this, we first attempted to form protoplasts from *M. rosaria* and to regenerate viable cells from protoplasts. Protoplasts were readily formed by growing cells in TS broth plus 0.2% glycine as described previously[19,20]. However, the resultant protoplasts did not regenerate viable cells when plated on modified R2 agar[19] in soft overlays, a procedure generally suitable to obtain regeneration of streptomycete protoplasts[20]. Instead, the protoplasts apparently underwent several cycles of cell division and produced microcolonies consisting primarily of osmotically fragile protoplasts. Since *M. rosaria* protoplasts were viable on modified R2 agar, we plated them on modified R2 agar and spotted different nutrients on the soft agar overlay and looked for a stimulation of cell regeneration. We found that the addition of mannitol caused a marked stimulation of cell regeneration, and in subsequent experiments demonstrated that the substitution of 10.3% mannitol for sucrose resulted in up to ~100% regeneration of cells from protoplasts. It was noted previously that the addition of mannitol to the regeneration agar R2YE caused improved regeneration of cells from protoplasts of *Nocardia mediterranei* and from protoplasts of several other ansamycin-producing *Nocardia* and *Streptomyces* species[21]. Thus our observations, coupled with those of SCHUPP and DIVERS[21], extend the utility of mannitol as an osmotic stabilizer to at least one species of *Micromonospora*, and suggest that mannitol might be generally useful to obtain regeneration of cells from protoplasts of other actinomycetes.

We then attempted to transform *M. rosaria* protoplasts with plasmid pIJ702[22] prepared from *Streptomyces lividans* using a variety of different transformation conditions, and plated the protoplasts on modified R2 with mannitol (modified R2M). No transformants were obtained using the standard procedures for transforming *Streptomyces fradiae*[4] or *Amycolatopsis orientalis*[11]. Also, no transformants were obtained using the *S. fradiae* procedure, but varying the polyethylene glycol (PEG) concentration from 20 to 55%; varying the PEG molecular weight from 1,000 to 6,000; or varying the protoplast concentration from 3-fold concentrated to 3-fold diluted. The transformation procedure for *S. lividans*[1,2] also could not be used since *M. rosaria* did not grow in YEME plus sucrose medium. However, transformants resistant to 25 $\mu$g/ml of thiostrepton were obtained at a frequency of about 2 per $\mu$g of DNA under the following conditions: Cells were grown

at 29°C in TS broth[19] plus 0.2% glycine; protoplasts were prepared by incubating cells at 23°C in P medium[23] with 5 mg/ml lysozyme for about 3 hours; protoplasts were concentrated by 24-fold and mixed with DNA plus 20% PEG1000; and 0.1 ml or less of the protoplasts were plated on modified R2M. Transformation was verified by extracting the plasmid from a transformant by the procedure of KIESER[24] and digesting it with restriction endonuclease *Cla* I. A plasmid the size of pIJ702 containing only one *Cla* I site as expected for pIJ702 was obtained from the transformant, but not from the parental strain. Plasmid DNA from the transformant was used to transform the parental *M. rosaria*, and transformants were obtained at a frequency of $1.5 \times 10^3$ per $\mu$g of DNA. These results suggest that *M. rosaria* expresses both restriction and modification, as is common for streptomycetes[25,26]. While the transformation frequencies with DNA extracted from a heterologous host was low, our data suggest that less restricting mutants of *M. rosaria* might be obtained by mutation and selection for increased transformability by plasmid pIJ702 and perhaps by other plasmid vectors as has been demonstrated with the highly restricting *S. fradiae*[27]. Our results indicate that at least one streptomycete plasmid cloning vector can replicate in *M. rosaria* and suggest that others might also function to allow gene cloning to proceed in this important genus that is more distantly related to *Streptomyces*[15] than most or all of the other genera previously demonstrated to be transformable or transducible by streptomycete plasmids. The substitution of mannitol for sucrose in the regeneration medium is an important element of the procedure, and it will be interesting to see if the addition of this variable to those defined previously[1,2,4,11,12] will allow further application of streptomycete vectors and protoplast transformation to establish gene cloning in a wider array of actinomycete genera.

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## References

1) BIBB, M. J.; J. M. WARD & D. A. HOPWOOD: Transformation of plasmid DNA into *Streptomyces* at high frequency. Nature 274: 398~400, 1978
2) THOMPSON, C.J.; J.M. WARD & D.A. HOPWOOD: Cloning of antibiotic resistance and nutritional genes in streptomycetes. J. Bacteriol. 151: 668~677, 1982
3) BALTZ, R. H. & P. MATSUSHIMA: Advances in protoplast fusion and transformation in *Streptomyces*. Exper. Suppl. 46: 143~148, 1983
4) MATSUSHIMA, P. & R. H. BALTZ: Efficient plasmid transformation of *Streptomyces ambofaciens* and *Streptomyces fradiae* protoplasts. J. Bacteriol. 163: 180~185, 1985
5) ACEBAL, C.; V. RUBIO & G. MARQUEZ: A method to transform the $\beta$-lactam antibiotic producer *Streptomyces wadayamensis*. FEMS Microbiol. Lett. 35: 79~82, 1986
6) LAMPEL, J. S. & W. R. STROHL: Transformation and transfection of anthracycline-producing streptomycetes. Appl. Environ. Microbiol. 51: 126~131, 1986
7) GARCIA-DOMINGUEZ, M.; J. F. MARTIN, B. MAHRO, A. L. DEMAIN & P. LIRAS: Efficient plasmid transformation of the $\beta$-lactam producer *Streptomyces clavuligerus*. Appl. Environ. Microbiol. 53: 1376~1381, 1987
8) HOPWOOD, D. A.; T. KIESER, D. J. LYDIATE & M. J. BIBB: *Streptomyces* plasmids: Their biology and use as cloning vectors. *In* The Bacteria, Vol. IX: Antibiotic-producing *Streptomyces*. *Eds.*, S. W. QUEENER & L. E. DAY, pp. 159~229, Academic Press, New York, 1986
9) HOPWOOD, D. A.: Cloning and analysis of antibiotic biosynthetic genes in *Streptomyces*. *In* Biological, Biochemical and Biomedical Aspects of Actinomycetes. *Ed.*, G. SZABO *et al.*, pp. 3~14, Akademiai Kiabo, Budapest, 1986
10) SENO, E. T. & R. H. BALTZ: Structural organization and regulation of antibiotic biosynthesis and resistance genes. *In* Regulation of Secondary Metabolism in Actinomycetes. *Ed.*, S. SHAPIRO, CRC Press, Boca Raton, 1988, in press
11) MATSUSHIMA, P.; M. A. MCHENNEY & R. H. BALTZ: Efficient transformation of *Amycolatopsis orientalis* (*Nocardia orientalis*) protoplasts by *Streptomyces* plasmids. J. Bacteriol. 169: 2298~2300, 1987
12) YAMAMOTO, H.; K. H. MAURER & C. R. HUTCHINSON: Transformation of *Streptomyces erythraeus*. J. Antibiotics 39: 1304~1313, 1986
13) PIDCOCK, K. A.; B. S. MONTENECOURT & J. A. SANDS: Genetic recombination and transfor-

mation in protoplasts of *Thermomonospora fusca*. Appl. Environ. Microbiol. 50: 693~695, 1985

14) McHENNEY, M. A. & R. H. BALTZ: Transduction of plasmid DNA in *Streptomyces* and related genera by bacteriophage FP43. J. Bacteriol. 170 (5): 1988, in press

15) STACKEBRANDT, E.; W. LUDWIG, E. SEEWALDT & K.-H. SCHLEIFER: Phylogeny of sporeforming members of the order *Actinomycetales*. Int. J. Syst. Bacteriol. 33: 173~180, 1983

16) BALTZ, R. H.: Genetics and biochemistry of tylosin production: A model for genetic engineering in antibiotic-producing *Streptomyces*. *In* Genetic Engineering of Microorganisms for Chemicals. *Ed.*, A. HOLLAENDER, pp. 431~444, Plenum Publishing Corp., New York, 1982

17) BALTZ, R. H.; J. T. FAYERMAN, T. D. INGOLIA & R. N. RAO: Production of novel antibiotics by gene cloning and protein engineering. *In* Protein Engineering. *Eds.*, M. INOUYE & R. SARMA, Academic Press, New York, 1986

18) GANGULY, A. K.; B. K. LEE, Y.-T. LIU, J. LOTVIN, O. SARRE & R. VAUGHN: Mutasynthesis of 23-*O*-mycinosyl-12,13-desepoxy-12,13-didehydrorosaramicin from tylosin. J. Chem. Soc. Chem. Commun. 1982: 855~857, 1982

19) BALTZ, R. H.: Genetic recombination in *Streptomyces fradiae* by protoplast fusion and cell regeneration. J. Gen. Microbiol. 107: 93~102, 1978

20) BALTZ, R. H. & P. MATSUSHIMA: Protoplast fusion in *Streptomyces*: Conditions for efficient genetic recombination and cell regeneration. J. Gen. Microbiol. 127: 137~146, 1981

21) SCHUPP, T. & M. DIVERS: Protoplast preparation and regeneration in *Nocardia mediterranei*. FEMS Microbiol. Lett. 36: 159~162, 1986

22) KATZ, E.; C. J. THOMPSON & D. A. HOPWOOD: Cloning and expression of the tyrosinase gene from *Streptomyces antibioticus* in *Streptomyces lividans*. J. Gen. Microbiol. 129: 2703~2714, 1983

23) OKANISHI, M.; K. SUZUKI & H. UMEZAWA: Formation and reversion of streptomycete protoplasts: Cultural condition and morphological study. J. Gen. Microbiol. 80: 389~400, 1974

24) KIESER, T.: Factors affecting the isolation of ccc DNA from *Streptomyces lividans* and *Escherichia coli*. Plasmid 12: 19~36, 1984

25) COX, K. L. & R. H. BALTZ: Restriction of bacteriophage plaque formation in *Streptomyces* spp. J. Bacteriol. 159: 499~504, 1984

26) CHATER, K. F.: *Streptomyces* phages. *In* The Bacteria, Vol. IX: Antibiotic-producing *Streptomyces*. *Eds.*, S. W. QUEENER & L. E. DAY, pp. 119~158, Academic Press, New York, 1986

27) MATSUSHIMA, P.; K. L. COX & R. H. BALTZ: Highly transformable mutants of *Streptomyces fradiae* defective in several restriction systems. Mol. Gen. Genet. 206: 393~400, 1987