Notes

pFJ269, A NEW PLASMID ISOLATED FROM A β-LACTAM ANTIBIOTIC PRODUCING STREPTOMYCETE

MICHAEL D. JONES
and JEFFREY T. FAYERMAN*

Lilly Research Laboratories,
Eli Lilly and Company
Indianapolis, Indiana 46285, USA

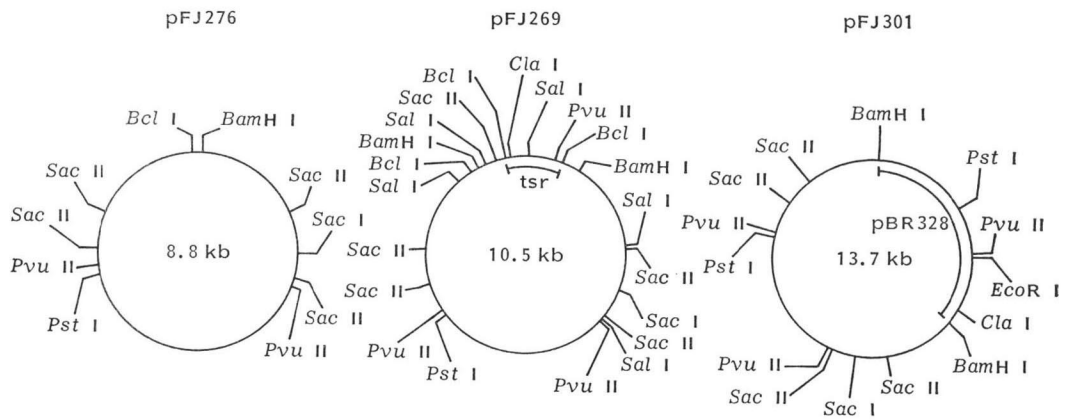
(Received for publication July 9, 1984)

Streptomyces are Gram-positive, filamentous bacteria which have a complex life cycle that includes formation of substrate and aerial mycelia and spores. *Streptomyces* produce approximately 70% of all naturally occurring antibiotics[1,2] and it is in the commercial production of antibiotics that this genus has been exploited. While the genetics and molecular biology of many *Streptomyces* are poorly understood, recent improvements in protoplast preparation, regeneration, fusion, and transformation[3~6] have facilitated the study of genetic organization, gene expression, and gene regulation in *Streptomyces*. Development of broad host range plasmid mediated gene cloning systems has also aided molecular studies in *Streptomyces*. Although plasmid vectors currently available appear to have utility in many *Streptomyces* species[7~9], restriction barriers between species and the possible instability of some plamids in a certain species could result in the need for different plasmid replicons. We report here the isolation and partial characterization of a new 8.8 kilobase plasmid from a β-lactam antibiotic producing streptomycete and its initial development into a gene cloning vehicle.

Total cellular DNA was isolated from *Streptomyces* strain A57284, an uncharacterized β-lactam producer, by procedures cited in RICHARDSON *et al.*[8]. Horizontal agarose gel electrophoresis revealed the presence of covalently closed circular DNA (cccDNA). The cccDNA was designated pFJ276. Although the strain from which pFJ276 was isolated produces a β-lactam antibiotic, no β-lactamase activity was detected in crude cell extracts. A restriction map of pFJ276 (Fig. 1) was constructed following digestion with several restriction endonucleases in various combinations. A 1.7 kilobase *Bam*H I fragment containing the *Streptomyces azureus* gene conferring resistance to thiostrepton, described by THOMPSON *et al.*[6], subcloned in our laboratory, was ligated to *Bam*H I digested pFJ276. The

Fig. 1. Restriction endonuclease cleavage maps of pFJ276, pFJ269 and pFJ301.

pFJ276 was isolated from strain A57284. pFJ269 was constructed by cloning a 1.7 kb *Bam*H I fragment containing the *S. azureus* gene conferring resistance to thiostrepton into the unique *Bam*H I site of pFJ276. PFJ301 is a chimeric plasmid comprised of pBR328[10] and pFJ276, and can replicate and be amplified in *E. coli*. Many of the restriction sites of the pBR328 portion of pFJ301 have been omitted.



ligated DNA was used to transform *Streptomyces ambofaciens* protoplasts by the method cited in JONES *et al.*[9]. Transformants were selected for resistance to 50 μg/ml thiostrepton. The recombinant plasmid pFJ269 (Fig. 1) was found among the thiostrepton resistant transformants from which plasmid DNA was isolated. The copy number of pFJ269 in *S. ambofaciens* after 48 hours of growth in liquid culture was estimated by the method cited in JONES *et al.*[9] to be about 40 copies per chromosome. Preliminary host range studies indicate that pFJ269 can function in *S. ambofaciens* and *Streptomyces* strain A49839, an uncharacterized β-lactam antibiotic producer that is different from *Streptomyces* strain A57284, the source of pFJ276. pFJ269 transforms *S. ambofaciens* at a frequency of about 10^3 to 10^4 transformants/μg. The initial transformation frequency of *Streptomyces* strain A49839 by pFJ269 was less than 50/μg because transformation procedures have not been optimized for this strain. The plasmid pFJ301 (Fig. 1) was constructed to serve as a convenient *Escherichia coli* source of pFJ276 by cloning pBR328 into the unique *Bam*H I site of pFJ276.

pFJ269 is the first cloning vehicle with a replicon isolated from a β-lactam antibiotic producing streptomycete. Although it is at an early stage of development, pFJ269 is an example of an alternative plasmid replicon that could be useful in fundamental and applied studies in *Streptomyces*.

Acknowledgments

The authors wish to thank Mrs. C. A. ALEXANDER for typing the manuscript and Mrs. N. E. MALIN for technical assistance.

References

1) HOPWOOD, D. A. & M. J. MERRICK: Genetics of antibiotic production. Bacteriol. Rev. 41: 595~635, 1977
2) HOPWOOD, D. A. & K. F. CHATER: Fresh approaches to antibiotic production. Phil. Trans. R. Soc. Lond. B209: 313~328, 1980
3) BALTZ, R. H.: Genetic recombination in *Streptomyces fradiae* by protoplast fusion and cell regeneration. J. Gen. Microbiol. 107: 93~102, 1978
4) HOPWOOD, D. A. & H. M. WRIGHT: Factors affecting recombinant frequency in protoplast fusions of *Streptomyces coelicolor*. J. Gen. Microbiol. 111: 137~143, 1979
5) BALTZ, R. H. & P. MATSUSHIMA: Protoplast fusion in *Streptomyces*: Conditions for efficient genetic recombination and cell regeneration. J. Gen. Microbiol. 127: 137~146, 1981
6) THOMPSON, C. J.; J. M. WARD & D. A. HOPWOOD: DNA cloning in *Streptomyces*: Resistance genes from antibiotic producing species. Nature 286: 525~527, 1980
7) MARTÍN, J.-F. & J. A. GIL: Cloning and expression of antibiotic production genes. Biotechnology 2: 63~71, 1984
8) RICHARDSON, M. A.; J. A. MABE, N. E. BEERMAN, W. M. NAKATSUKASA & J. T. FAYERMAN: Development of cloning vechicles from the *Streptomyces* plasmid pFJ103. Gene 20: 451~457, 1982
9) JONES, M. D.; J. A. MABE, W. M. NAKATSUKASA & J. T. FAYERMAN: pFJ265, a new cloning vehicle for *Streptomyces*. Plasmid 11: 92~95, 1984
10) WEST R. W., Jr. & R. L. RODRIGUEZ: Construction and characterization of *Escherichia coli* promotor-probe plasmid vectors. III. pBR322 derivatives with deletions in the tetracycline-resistance promotor region. Gene 20: 291~304, 1982