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Properties of Recombinant Derivatives of pJY501, A Multi-copy *Streptomyces* plasmid

Yum, Do-Young, In-Soo Kong and Ju-Hyun Yu*

Department of Food Engineering, College of Engineering, Yonsei University, Seoul 120-749, Korea

Multi-copy *Streptomyces* 플라스미드, pJY501 의 재조합 유도체의 특성

염도영 · 공인수 · 유주현*

연세대학교 공과대학 식품공학과

The restriction cleavage map of multi-copy recombinant plasmid, pJY502 (5.5 kb), carrying the thiostrepton resistance gene (*tsr*) was determined. Comparison of the restriction pattern with that of *Streptomyces* plasmids previously demonstrated that pJY502 was novel. The plasmid pJY502 had a broad host range in *Streptomyces* and contained single *Bgl*II site for cloning purpose. Transformation frequency of pJY502 was 2.2×10^5 in *S. lividans*. *E. coli-Streptomyces* bifunctional plasmid, pJY504, was also constructed.

Streptomyces species are Gram-positive multicellular organisms of industrial importance. Over 70% of the naturally occurring antibiotics are produced by these organisms (1). Recent developments in *Streptomyces* genetics have opened the possibility of cloning genes and improving the strains (2, 3). An important development in *Streptomyces* genetics has been the isolation and characterization of *Streptomyces* plasmids as potential vectors. For the application of cloning in *Streptomyces,* in application of specific genes or expression of genes in different hosts, plasmids and derivatives of high copy number and their broad host range have been reviewed. Several high copy number plasmid, pIJ101 from *Streptomyces lividans* ISP5434 (4), pUC6 from *S. espinosus* (5) and pJV1 from *S. phaeochromogenes* (6, 7) were described and their derivatives were constructed as useful vectors. We are also working toward the development of a plasmid-mediate cloning system in *Streptomyces.*

Key words: *Streptomyces,* plasmid vector, broad host range
*Corresponding author

In this report, we describe the characteristics of small and multi-copy derivatives of pJY501 (8) and compare with useful cloning vectors already in use.

Materials and Methods

Bacterial strains and plasmids

The pJY501 and the recombinant plasmid pJY502 carrying the thiostrepton resistance gene (*tsr*) that had already been studied by the present authors (8) was used throughout this study. *S. lividans* TK24 (9) used as the standard host and pIJ702 (10) and pIJ41 (11) were donated by Professor D.A. Hopwood, Johe Innes Institute, Norwich, UK. Other *Streptomyces* strains used for host range studies are listed on Table 1. *E. coli* HB101 (12) was used as host for pBR322 (13) and bifunctional derivatives.

Culture condition and transformation procedure

All *Streptomyces* for transformation experiments were grown in YEME medium containing 34% sucrose, 0.2% $MgCl_2 \cdot 6H_2O$ and 0.5% glycine. Protoplast formation and transformation of *S. lividans*

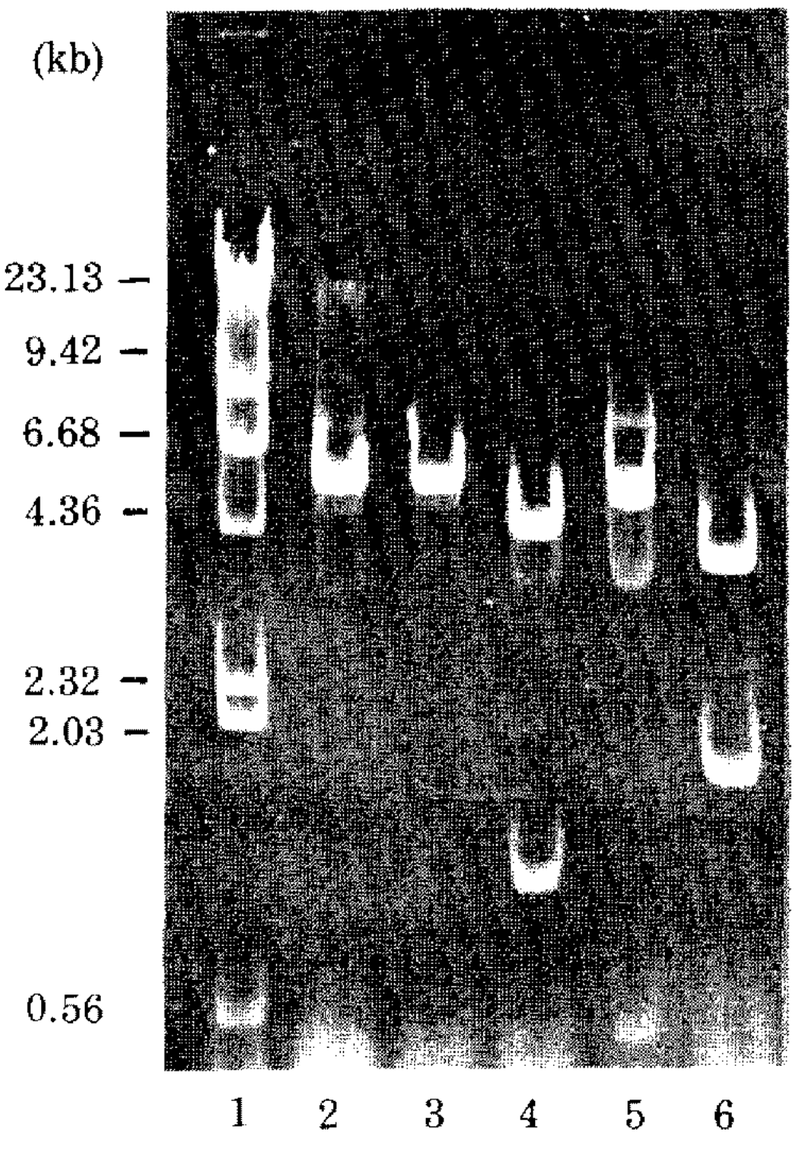


Fig. 1. Agarose gel electrophoresis of plasmid DNA pJY502 digested by various restriction enzymes.
lane 1: λ DNA (*Hin*dIII), lane 2: pJY502 (*Bgl*II), lane 3: pJY502 (*Bam*HI), lane 4: pJY502 (*Bcl*I), lane 5: *Kpn*I, lane 6: *Pvu*II

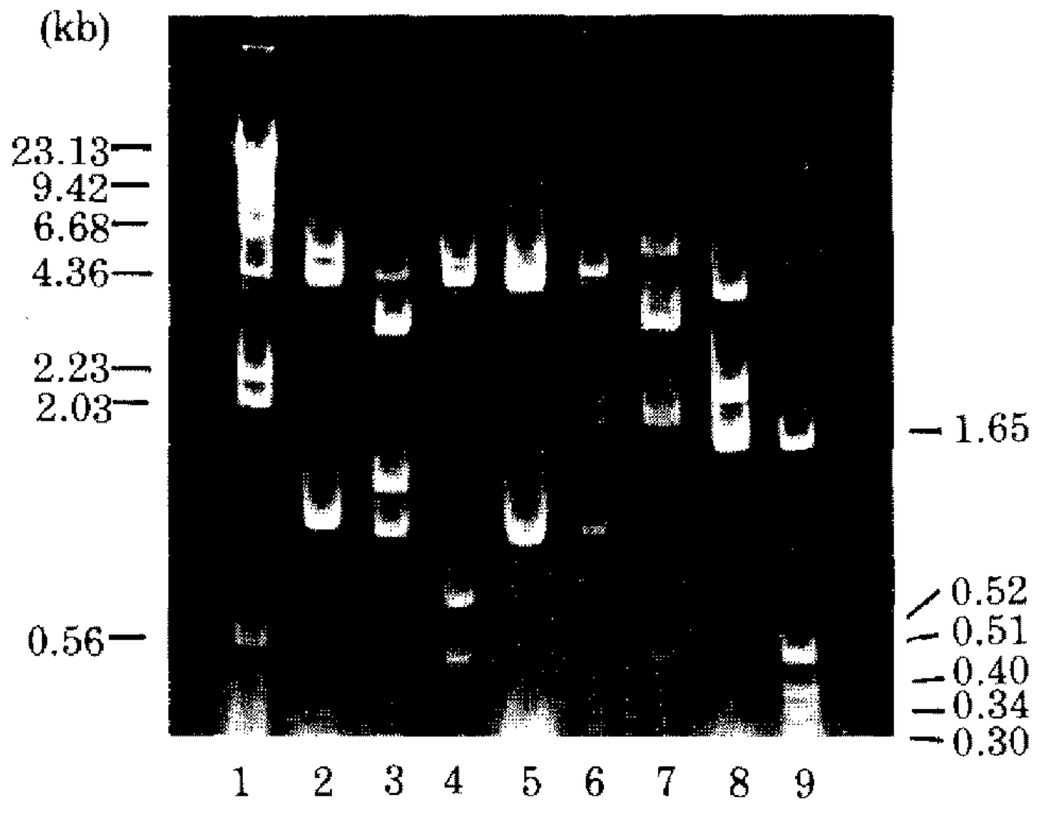


Fig. 2. Agarose gel electrophoresis of double digested plasmid DNA pJY502.
lane 1: λ DNA (*Hin*dIII), lane 2: pJY502 (*Bam*HI & *Bgl*II), lane 3: pJY502 (*Bam*HI & *Bcl*I), lane 4: pJY502 (*Bam*HI & *Kpn*I), lane 5: pJY502 (*Bcl*I & *Bgl*II), lane 6: pJY502 (*Bcl*I & *Kpn*I), lane 7: pJY502 (*Kpn*I & *Bgl*II), lane 8: pJY502 (*Pvu*II & *Bam*HI), lane 9: pJY322 (*Hin*fI).

were as in Thompson *et al.* (14). Direct selection for thiostrepton resistant transformants was made by overlaying regeneration medium (R2YE) with 2.5 m*l* soft Nutrient agar (Difco) containing 500 μg thiostrepton per m*l* after incubation for 20 hrs (4). Thiostepton (kindly donated by Mr. S.J. Lucania of E.R. Squibb and Son, New Brunswich, NJ, USA) was used in liquid at 5 μg per m*l* and solid media at 50 μg per m*l*. *E. coli* HB101 was transformed as described by Nogard *et al.* (15).

DNA manipulation

Plasmid isolation, restriction enzyme digestion, bacterial alkaline phosphatase treatment, agarose gel electrophoresis and ligation of DNA were essentially as Hopwood *et al.* (16).

Result

Restriction endonuclease cleavage map of pJY502

The restriction cleavage map of pJY502 were determined from cleavage patterns obtained by successive digestion with various combination of restriction enzymes (Fig. 1-3). Comparison of the restriction pattern with those of plasmids (i.e., SCP2*, SLP1.2 (17), pIJ101 (4), pUC6 (5), pSK1 and pSK2 (18), pFJ103 (19) and pJV1 (6)) previously demonstrated that the isolated plasmid from soil, pJY501 (8), was novel.

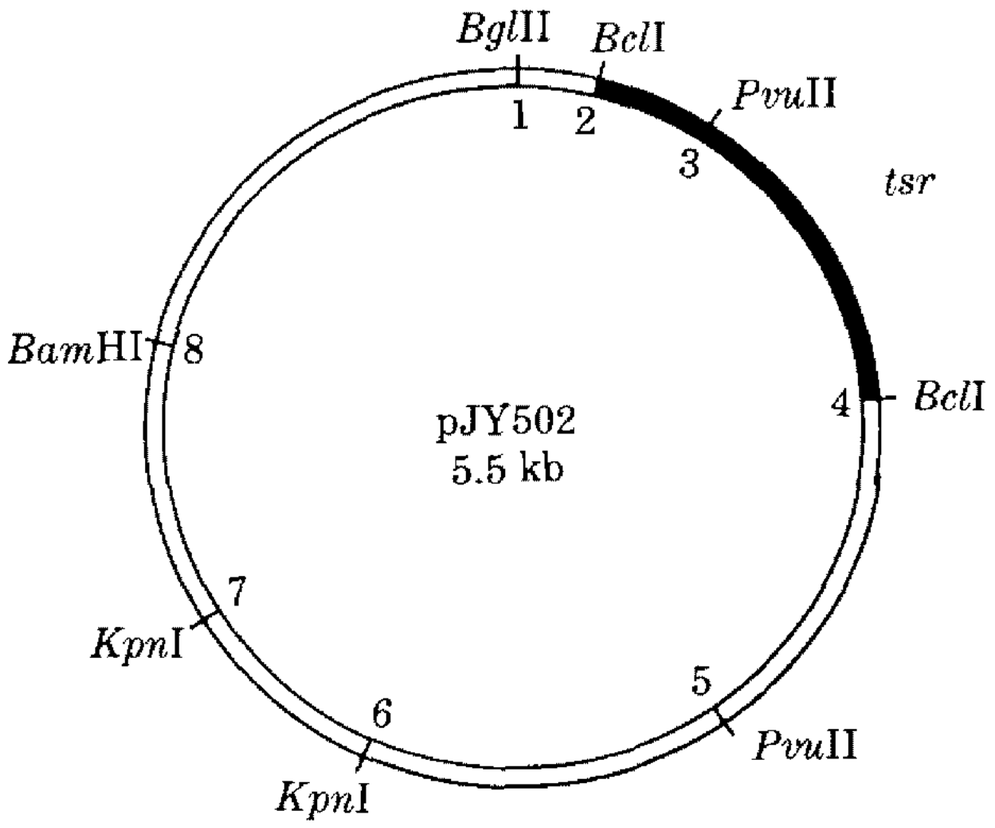
Lethal zygosis and compatibility of pJY501 with other plasmids

The known plasmids of *Streptomyces* exhibit a property that have been called lethal zygosis co-related with plasmid transfer (9). We could detect a lethal zygosis in regenerating *S. lividans* lawns after attempted transformation with pJY501.

The compatibility of pJY501 and pIJ702 (having the replicon of pIJ101) was tested by transformation of *S. lividans* with pJY501 and pIJ702 and isolation of thiostrepton resistant colonies containing both pJY501 and pIJ702. One such colony was non-selectively subcultured and replated again non-selectively to give single colony. A total of 10 colonies tested contained both pJY501 and pIJ702, and two plasmids showed same apparent copy number on agarose gel. Also pJY502 was compatible with pIJ41 containing the essential region of SLP1.2 (14). Therefore, the result showed that two families of high-copy plasmids are compatible.

Host range of pJY502

The transfer of pJY502 to *Streptomyces* and



Distance from *Bgl*II site (kb)

1. 0.0	4. 1.3	7. 3.6
2. 0.2	5. 2.2	8. 4.3
3. 0.5	6. 3.1	1. 5.5

Fig. 3. Restriction map of pJY502.
No sites found for *Pst*I, *Eco*RI, *Hin*dIII, *Xba*I, *Xho*I and *Sst*I.

Table 1. Transformation of pJY502 into various *Streptomycetes*.

Strain	Transformation by pJY502*
S. albus subsp. *albus* ATCC29795	Low**
S. antibioticus IFO12838	High
S. aureus ATCC21428	–
S. aureus ATCC21658	Low
S. avermitilis ATCC31267	Low
S. cacaoi subsp. asoensis IFO13813	Low
S. coelicolor IAM1023	High
S. flaveolus IFO3715	High
S. griseus IFO12875	–
S. griseus subsp. *griseus* IFO13189	–
S. hygroscopicus ATCC21705	–
S. kasugaensis ATCC15715	Low
S. lividans	High
S. mediocidicus ATCC13278	–
S. mitakaensis ATCC15295	High
S. tandae ATCC31160	–
N. mediterranei IFO13142	–

*pJY502 DNA was isolated from *S. lividans*.
**Low, lower than 10^3 transformants per μg plasmid DNA: High, higher than 10^3 transformants per μg plasmid DNA.

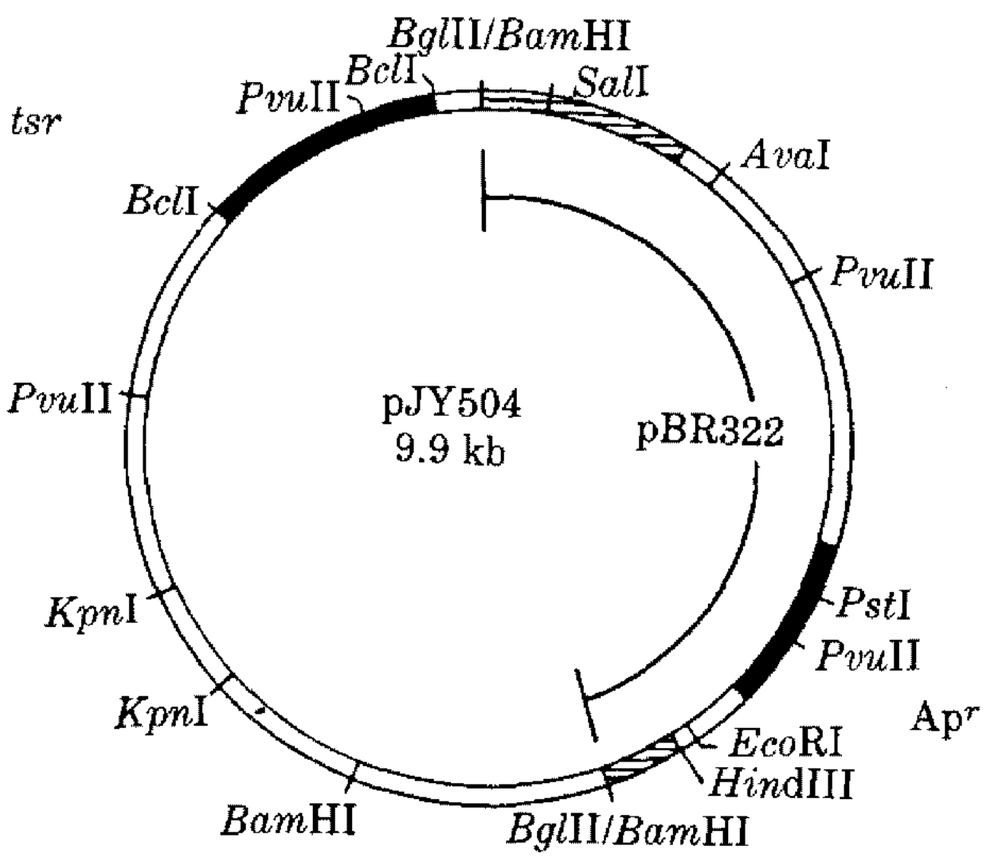


Fig. 4. Restriction map of pJY504.

Nocardia species was attempted. Selection for thiostrepton resistance was made by overlaying the regenerating protoplasts after 20 hrs with soft agar containing thiostrepton. The results are shown in Table 1. No attempt was made to optimize transformation and regeneration conditions for each hosts; the procedure developed for *S. lividans* were used throughout (14). The plasmid pJY502 had a broad host range in *Streptomyces*. The confirmation of transformation was made in each case by isolation of plasmid DNA, except in the case of *Nocardia mediterranei* IFO13142 where partially thiostrepton-resistant colonies were obtained on transformation with pJY502 but no plasmid DNA could detected by DNA isolation. The low frequencies observed or several species could be explained either by the non-optimal procedures for protoplast preparation and transformation or by host restriction systems (7).

Construction of bifunctional plasmids

The plasmid pBR322 was digested with *Bam*HI and ligated with *Bgl*II digested pJY502. *E. coli* HB101 was transformed with each of the ligated DNA. The recombinant plasmids were found in *E. coli* HB101 by isolation of plasmid DNA of ampicillin resistant, tetracycline sensitive transformants. *E. coli-Streptomyces* bifunctional plasmid, pJY504, had pBR322 in the *Bgl*II site of pJY502 (Fig. 4). Transformation frequency of pJY504 was 1.8×10^3 in *S. lividans*, 4.2×10^3 in *E. coli*. The observation that pJY504 could transform both *Streptomyces* and E. coli indicated that this vector could be used to

clone genes from both organisms. Also this experiment suggested that the *Bgl*II site of pJY502 was not in essential region, and that *Bgl*II useful for cloning with the widely used *Mbo*I (*Sau*3AI) was potential cloning site in pJY502.

Discussion

Comparison of restriction enzyme pattern of the *tsr*-containing derivative demonstrated that the plasmid pJY501 was new. The compatibility of pJY502 with pIJ702 could be very useful in examining the effect of combinations of independently cloned genes within the same cell, without the need for further *in vitro* manipulation. The plasmid was readily manipulated and stable without deletion in *Streptomyces*. Their *tsr* containing derivative, pJY502, was stable without rearrangement and had wide species host range in *Streptomyces*. The *Bgl*II site of pJY502 in experiment of construction of *E. coli-Streptomyces* bifunctional plasmid was proven as non-essential site useful for cloning of *Mbo*I generated fragments. Because of their originally small size, high copy number and broad host range, pJY501 and their derivatives are attractive potential vectors for cloning in *Streptomyces*. We think that more useful vectors could be developed if experiments for allocation of non-essential regions of plasmids and size reduction are performed. We wish to appreciate Professor D.A. Hopwood for helpful discussion and generous gifts of *S. lividans* strains.

요 약

Thiostrepton 내성 유전자(*tsr*)를 포함하는 multicopy 재조합 플라스미드 pJY502(5.5kb)의 제한효소 지도를 비교해본 결과 pJY502는 새로운 플라스미드로 확인되었다. pJY502는 *Streptomyces*에서 넓은 host range를 나타내었으며 cloning에 사용할 수 있는 단일 *Bgl*II 제한효소 인식부위를 갖고 있었다. pJY502의 형질전환 빈도는 *S. lividans*에서 2.2×10^5이었다. 또한 *E. coli-Streptomyces* bifunctional 플라스미드 pJY504을 제조하였다.

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