Note

Expression of the Aminoglycoside 6-Adenylyltransferase Coding Gene from *Bacillus subtilis* in *Escherichia coli*

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It has been reported that expression of tetracycline- or chloramphenicol-resistance genes that originated from Gram-positive bacteria were less effective in *Escherichia coli* than in *Bacillus subtilis*.¹⁻⁴⁾ A useful selection marker which is expressed distinctly in both Gram-positive and -negative bacteria has not been found.

We have reported that derivatives of the Gram-positive bacterium *B. subtilis* Marburg 168, susceptible to streptomycin (SM), produce a chromosomally encoded aminoglycoside 6-adenylyltransferase [AAD(6)],^{5,6)} and the nucleotide sequence of the AAD(6) coding gene, designated *aadK*, has been determined.⁷⁾

This report describes the usefulness of the cloned *aadK* gene as an SM-resistance marker for a cloning vector in *E. coli* and *B. subtilis*.

The 2.7-kb *Hind*III fragment containing the *aadK* gene derived from *B. subtilis* Marburg 168 was cloned into plasmid pACYC184.⁸⁾ Two types of the recombinant plasmids carrying the 2.7-kb fragment with an opposite orientation, pTZ331⁷⁾ and pTZ332, were obtained in *E. coli* transformants. The pTZ331 DNA could retransform *E. coli* at a frequency of about 10⁵ transformants per µg of DNA. To confirm the origin of the cloned *aadK* gene, Southern hybridization⁹⁾ of the 2.7-kb fragment was done with *Hind*III-digested chromosomal DNA from *B. subtilis* Marburg 168, BD224 and ATCC6633. The 2.7-kb fragment hybridized with a single *Hind*III-cleaved chromosomal fragment from both *B. subtilis* Marburg 168 and its derivative strain BD224, but not with that from the wild type strain ATCC6633, which is usually used as a test strain in the microbioassay to measure the potency of antibiotics (data not shown).

To investigate the expression of this gene in *B. subtilis*,

plasmids pTZ333 and pTZ336 were constructed by insertion of the 2.7-kb fragment into the unique *Hind*III site of vector pTP4¹⁰⁾ with an opposite orientation (Table I), and these plasmids were used to transform the wild type strain ATCC6633, which do not carry the *aadK* gene on the chromosome, and the recombination-deficient strain BD224. All transformants tested harbored the intact plasmid pTZ333 or pTZ336 after 100 generations in the absence of selection for SM-resistance. When the pTZ333 and pTZ336 isolated from *B. subtilis* BD224 were used to retransform *B. subtilis* ATCC6633, the transformants were obtained at a frequency of about 10⁴ cells per µg of DNA. The frequency with pTZ333 or pTZ336 was nearly of the same order of magnitude as that of pTP4. The plasmid copy numbers,¹¹⁾ as measured for pTZ331 in *E. coli* C600

Table I. SUSCEPTIBILITY OF STRAINS TO STREPTOMYCIN

Strain	MIC of SM (µg/ml)	Specific enzymatic activity of AAD(6) ^a
<i>E. coli</i>		
C600	3.13	0 ^b
C600 (pTZ331)	400	35.50
C600 (pTZ332)	800	34.30
<i>B. subtilis</i>		
Marburg 168	12.5	1.44
BD224	3.13	1.81
ATCC6633	3.13	0 ^b
BD224 (pTZ333)	100	10.95
BD224 (pTZ336)	100	12.78
ATCC6633 (pTZ333)	100	16.07

DNA manipulations were essentially as described by Maniatis *et al.*¹⁴⁾ Transformation of *E. coli* C600 (*thi-1*, *thr-1*, *leuB6*, *lacY1*, *tonA21*, *supE44*) was done by the calcium shock procedure.¹⁵⁾ Competent cells of *B. subtilis* BD224 (*trpC2*, *thr5*, *recE4*) were made by the method of Contente and Dubnau.¹⁶⁾ Protoplast cells of *B. subtilis* ATCC6633 were transformed by the method of Chang and Cohen.¹⁷⁾ Transformants were selected using LB agar plates or DM3 plates containing 25 µg of SM per ml. Minimum inhibitory concentrations (MICs) were measured by the agar dilution method published by the Japan Society of Chemotherapy.¹⁸⁾ Inactivation of SM was done by the method of Kono *et al.*⁵⁾ The reaction mixture consisted of 0.3 ml of crude extract, 0.2 ml of 40 mM ATP in TMK buffer (pH 7.8) and 12.5 µl of 1 mM SM. The residual activity in the reaction mixture was measured using *B. subtilis* ATCC6633 as a test organism. The protein concentration was measured by the method of Lowry *et al.*¹⁹⁾

^a Expressed as nanomoles of SM inactivated per hour per mg of protein.

^b 0: less than 0.005.

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and for pTZ333 in *B. subtilis* BD224,¹²⁾ were 25 and 50 per chromosome, respectively.

We found during our cloning experiments that expression of SM-resistance in *E. coli* was 4 to 8-fold higher than in *B. subtilis* (Table I).

We postulated that the *aadK* gene would be a useful selection marker for a shuttle vector for the follow-up reasons: (i) the *aadK* gene expressed SM resistance distinctly in both *E. coli* and *B. subtilis*; (ii) recombinant plasmids carrying the *aadK* gene were quite stable; (iii) the high activity of AAD(6) could be detected by the simple method of Kono *et al.*^{5,13)}; and (iv) the *aadK* gene had single *KpnI* and *BglII* sites within the coding region, which allowed inactivation by insertion of foreign DNA fragments at these sites.

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