NEGATIVE CONTROL FOR THE EXPRESSION OF STREPTOMYCIN RESISTANCE GENE FROM STREPTOMYCIN-PRODUCING *STREPTOMYCES GRISEUS*

Sir:

We have cloned a DNA segment from *Streptomyces griseus* ISP 5236 which directs streptomycin (SM) phosphotransferase (sph), an inactivating enzyme that mediates SM resistance, into the plasmid pIJ702[1] in *Streptomyces lividans*.[2] Further investigation of the cloned DNA (7.0 kb *Bgl* II fragment) revealed the existence of a gene which negatively controls expression of the sph gene and is located in the region adjacent to the sph gene. The negative control was derepressed by SM. This communication deals with the negative control of sph gene expression.

As shown in Fig. 1, the sph gene was determined to be in the *Bam*H I (3)-*Sph* I (3) region and to be transcribed from the *Sph* I (3) site toward the *Bam*H I (3) site by sub-cloning and nucleotide sequence analysis (details will be published elsewhere).

Four plasmids were constructed from plasmid pST141 containing the 7.0 kb *Bgl* II fragment. One, pHT000, was obtained by deletion of the *Sph* I (1)-*Sph* I (2) and *Sph* I (3)-*Bgl* II (2) regions followed by sub-cloning into the *Bgl* II-*Sph* I site of pIJ702 vector. The *Bam*H I (3)-*Sph* I (3) segment was then cut out from pHT000 and sub-cloned into pIJ702 as pHT008. The third plasmid, pHT002, was constructed by sub-cloning the *Sph* I (2)-*Pst* I fragment of pST141 into the *Sph* I-*Pst* I site of pIJ702. In addition, to change the order of *Sph* I (2)-*Pst* I and *Bam*H I (3)-*Sph* I (3) regions of pHT000, the *Bam*H I (3)-*Sph* I (3) segment was cut out from pHT000 and sub-cloned into the *Bgl* II-*Sph* I site of pHT002 as pHT010.

The four strains of *S. lividans* thus obtained were grown at 27°C in YEME[3] medium. In the late log-phase of their growth, two volumes of fresh YEME were added to the cultures, which were then divided into three portions. SM or streptidine (5 μg/ml) was added to two of the portions; the third was not supplemented. Incubation was continued overnight. The mycelium from each of the three cultures was washed with buffer (10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NH_4Cl, 3 mM 2-mercaptoethanol, pH 7.6). The washed mycelium was disrupted by sonication

Fig. 1. Plasmid constructions.

Solid lines represent the inserted DNA. pST141 shows the originally cloned DNA fragment (7.0 kb) with restriction sites. The sph gene is indicated by an arrow.

||: Termination of transcription.

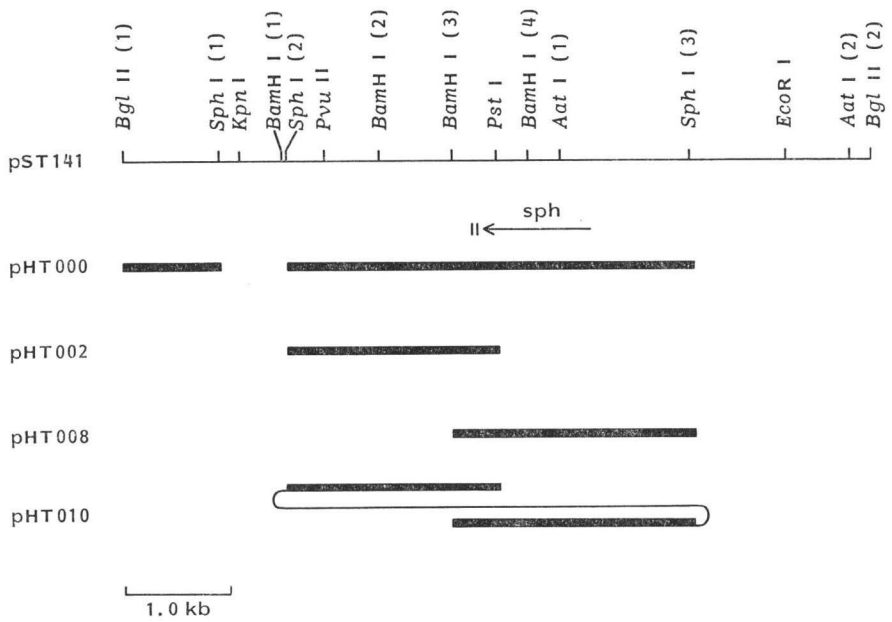

Table 1. Specific activities of sph.

Plasmid	SM-resistance (20 μg/ml)	Specific activity (μmol/mg/hour) of sph		
		None	+ SM	+ Streptidine
pHT000	+	<1	11	<1
pHT002	−	<1	<1	nd
pHT008	+	6	9	nd
pHT010	+	<1	6	nd

Specific activities of sph are expressed as μmol SM inactivated (mg protein)$^{-1}$hour^{-1} at 37°C in the buffer described in the text.

nd: Not determined.

after being suspended twice in its volume of buffer. After centrifugation at 30,000×*g* for 30 minutes at 4°C, the supernatant was used to assay for SM-inactivation and protein. The SM-inactivation assay mixture (50 μl) contained 100 mM Tris-maleic acid pH 7.0, 10 mM $MgSO_4$, 3 mM ATP, 1 mM SM and the supernatant, and was incubated at 37°C for 1 hour. Remaining SM was assayed by the agar-diffusion method.

As shown in Table 1, strains harboring pHT000 and pHT010 showed pronounced sph activity only when they were incubated with SM. On the other hand, the strain containing only pHT008 showed a significant level of sph activity with or without addition of SM. No change in its plasmid profile was detected. The strain harboring pHT-002 showed no sph activity even when SM was added to the medium. These results indicate that the sph gene is encoded in the *Bam*H I (3)-*Sph* I (3) region (2.3 kb), and that there is a negative control gene in the *Sph* I (2)-*Pst* I region (1.9 kb) (Fig. 1). Nucleotide sequence analysis revealed open reading frames in the above 2.3 kb and 1.9 kb fragments. The sph enzyme and a protein were purified from cell-free extract of *S. lividans* harboring pST141 by SM-affinity column chromatography. The amino-terminal sequences of the sph enzyme and protein (MW=35,000 and 40,000 from sodium dodecyl sulfate-polyacrylamide gel electrophoresis) corresponded with those predicted from nucleotide sequences of the 2.3 kb and 1.9 kb fragments, respectively (detailed data will be published elsewhere). Therefore the 1.9 kb fragment encodes the protein, probably a repressor to control expression of the sph gene in the 2.3 kb fragment. It is of interest that the sph gene was derepressed from the negative control by SM but not by streptidine, an intermediate of SM biosynthesis which is known to be phosphorylated by the sph enzyme.[4]

Recently, OHNUKI *et al.* reported that amidinotransferase and dihydrostreptosyltransferase genes which are involved in SM biosynthesis are clustered with the sph gene.[5] Based on their data, the negative control gene should be placed between the sph gene and the amidinotransferase gene. They concluded that there was a positive effector gene for the full expression of the sph gene and the amidinotransferase gene in the same gene cluster. The A-factor gene is also known to positively control expression of the sph gene.[6] Accordingly, it is obvious that expression of the sph gene is controlled in a complicated manner and it would be of interest to determine whether or not the negative control system is derepressed by A-factor or by the positive effector.

Correlation between self-resistance gene(s) and antibiotic biosynthesis genes has been suggested by the observation that the pattern of self resistance to an antibiotic correlates closely with the type of antibiotic produced. Recently, gene clusters containing both self-resistance gene(s) and antibiotic-biosynthesis genes were reported in an erythromycin producing strain[7] and a methylenomycin producing strain (M. BIBB, personal communication).[8] Moreover, our results in this report and OHNUKI *et al.*'s data[5] support the correlation among self-resistance, biosynthesis and their regulatory genes.

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