NUCLEOTIDE SEQUENCE OF Mec$^+$ GENE REGION OF *STREPTOMYCES KASUGAENSIS*

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The development of new host-vector systems in diverse species will be useful for breeding and gene cloning in *Streptomyces*. We have developed the host-vector system in *Streptomyces kasugaensis*, kasugamycin- and aureothricin-producer[1,2].

In the course of this study, DNA fragment containing Mec$^+$ gene which restores the nutritional mutation of *S. kasugaensis* G3 requiring both methionine and cystein for its growth was inserted into pSK2, one of the plasmids resident in *S. kasugaensis*, and into pIJ702[3], a plasmid of *S. lividans*, respectively. They were subcloned to elucidate the essential region of Mec$^+$ gene by *in vitro* deletion and self ligation. Finally, pSK21-TM6, pSK21-TM101 and pIJ702ΔSAlSP which carry thiostrepton-resistance gene as well as Mec$^+$ gene were obtained[4]. From these construction experiments, the Mec$^+$ gene was assigned to a fragment of *Sph* I$\sim$*Cla* I$\sim$*Sac* I (1.0 kb) in the inserted DNA[4].

Further analysis was done to make clear the location of the structural gene of Mec$^+$ gene using pIJ702-M1 (original Mec$^+$ recombinant of pIJ702). As shown in Fig. 1, a fragment containing *Bgl* II site was inserted into the *Cla* I site of Mec$^+$ region and the deletion and self ligation were carried out by the application of *Bgl* II sites located outside of Mec$^+$ gene. The clones were selected by thiostrepton resistance, and the complementation ability of Mec$^-$ was tested. The results suggested that the structural gene of Mec$^+$ gene located in the region (approximately 580 bp) between *Sph* I and *Cla* I site.

The determination of the nucleotide sequence of DNA segment including the *Sph* I$\sim$*Cla* I$\sim$*Sac* I region was carried out by the dideoxy method using M13 phage[5~7]. DNA fragments to be sequenced were cut off from pSK21-TM6 or -TM101 by the appropriate restriction

Fig. 1. Minimal region for Mec$^+$ expression.
Thick black bar indicates Mec$^+$ gene to be tested and white bar is the fragment inserted.

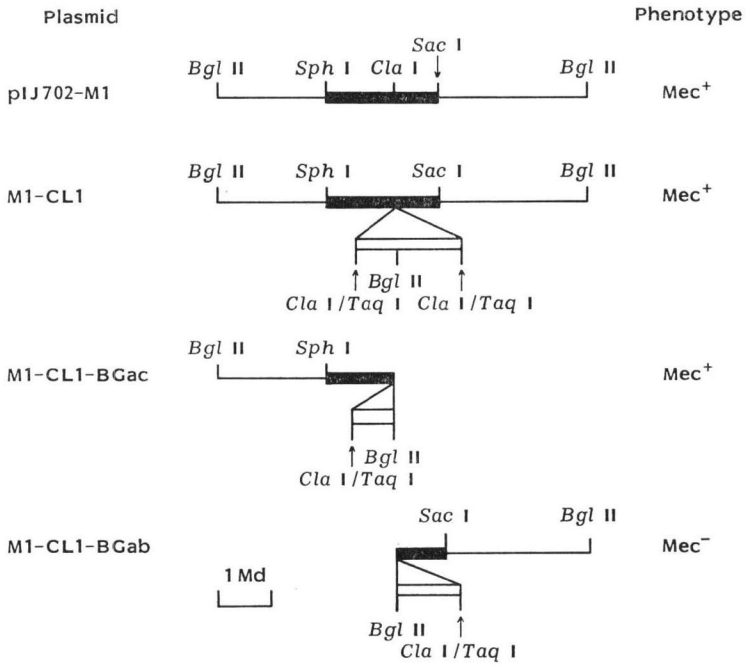


Fig. 2. Sequence analysis strategy for the region of Mec$^+$ gene.

Thin line indicates DNA fragment cloned into M13 phage and thick bar with arrow indicates the area sequenced and the direction.

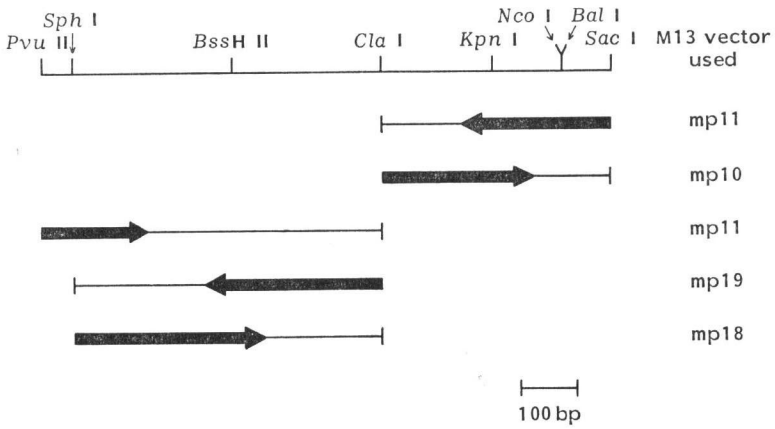


Fig. 3. Possible open reading frame in Mec$^+$ gene region.

Reading frame (a′) consists of white bar and thick black bar (reading frame (a)), and has GTG as initiation codon. Other reading frames start from ATG initiation codon.

Arrow indicates the direction of transcription.

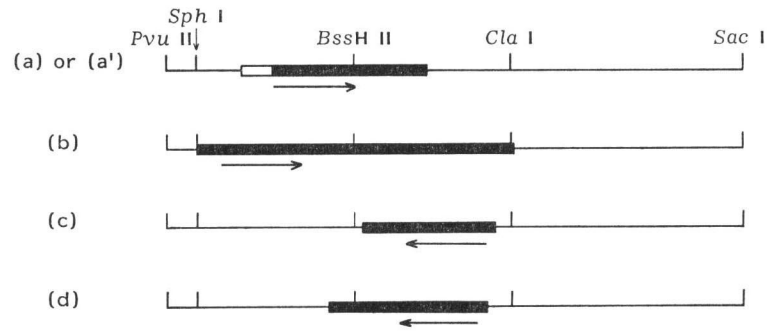


Table 1. Distribution of nucleotides in codon triplets of the possible reading frame.

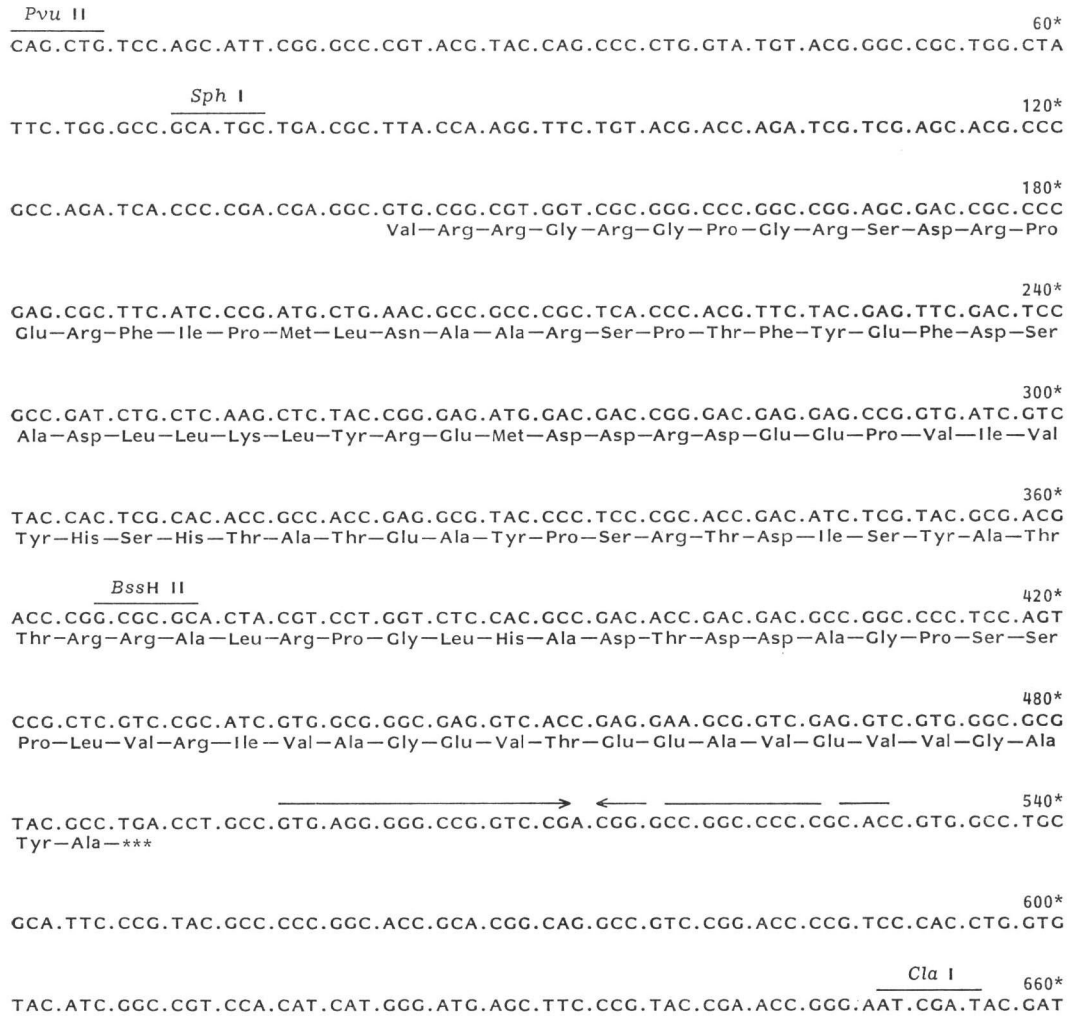
Reading frame	Total bases	G+C content (%)			
		Codon position			Overall
		1st	2nd	3rd	
(a)	291	69	47	91	69
(a′)	345	71	51	90	71
(b)	588	84	66	60	70
(c)	246	83	61	79	74
(d)	303	62	72	89	74

enzymes and then inserted into the corresponding cloning site of a set of M13 phage, namely mp10 and mp11 or mp18 and mp19, in both directions. Fig. 2 shows the cloning strategy and areas to be sequenced. The nucleotide sequence was determined (see Fig. 4. Sequence data of *Cla* I~*Sac* I region are not presented.). The average G+C content of this sequence was 71%, which was nearly close to that of *Streptomyces* genomic DNA (72~74%). From the examination of the distribution of start codon and stop codon in the region between *Sph* I and *Cla* I sites, four

Fig. 4. Nucleotide sequence of the region containing Mec$^+$ gene of *S. kasugaensis*.

Amino acid sequence predicted by nucleotide sequence is that of open reading frame (a′) described in the text.

Arrow bar indicates the inverted repeat sequence.



possible open reading frames for Mec$^+$ gene were selected as shown in Fig. 3.

Recently, on G+C distribution within codons of several *Streptomyces* gene, it was reported that mol % of G+C in the third codon position was extremely high (82~97%), and that in the second codon position was relatively low (43~51%)[8,9]. These facts were applied to determine the reading frame for Mec$^+$ gene, and as shown in Table 1, the G+C distribution in (a) or (a′) reading frame was found to be coincident with the tendency in *Streptomyces* genes mentioned above. The nucleotide and amino acid sequence of open reading frame (a′) are shown in Fig. 4. An inverted repeat sequence (17 bp) was found in 7 base pairs after stop codon of this reading frame. However, the presumed promoter and SD sequence were not found, likely to the case of *aph* gene of *S. fradiae* determined by THOMPSON *et al.*[9], though A+T content in upstream region preceding this reading frame was somewhat rich compared to other regions.

The protein encoded by Mec$^+$ gene was searched from the cell-free crude extracts of *S. kasugaensis* G3 carrying pSK21-TM101 and G3 strain itself grown in minimal medium, by means of disc gel electrophoresis. The protein less than 13,000 daltons which was assumed from

reading frame (a′) has not been detected yet.

This sequence of Mec$^+$ gene is the first nucleotide sequence of the gene related to the primary metabolism of *Streptomyces*.

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