NUCLEOTIDE SEQUENCE OF Mec+ GENE REGION OF STREPTOMYCES KASUGAENSIS

Kiyoshi Hirawasa, Masaru Ichihara and Masanori Okanishi

Department of Antibiotics, National Institute of Health, Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received for publication July 13, 1985)

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-producer1,2).

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 pIJ7023), 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 pIJ702dSAISP which carry thiostrepton-resistance gene as well as Mec+ gene were obtained4). From these construction experiments, the Mec+ gene was assigned to a fragment of Sph I ~ Cla I ~ Sac I (1.0 kb) in the inserted DNA4).

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 ~ Cla I region was carried out by the dideoxy method using M13 phage5~7). 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.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>pIJ702-M1</td>
<td>Mec+</td>
</tr>
<tr>
<td>M1-CL1</td>
<td>Mec+</td>
</tr>
<tr>
<td>M1-CL1-BGab</td>
<td>Mec+</td>
</tr>
<tr>
<td>M1-CL1-Tm6</td>
<td>Mec+</td>
</tr>
</tbody>
</table>

---

**Diagram:**

- **pIJ702-M1**
  - Bgl II
  - Sph I
  - Cla I
  - Sac I
  - Mec+

- **M1-CL1**
  - Bgl II
  - Sph I
  - Cla I
  - Mec+

- **M1-CL1-BGab**
  - Bgl II
  - Sph I
  - Cla I
  - Mec+

**Restriction Sites:**
- Bgl II
- Sph I
- Cla I
- Sac I

---

**Diagram:**

- **pIJ702-M1**
  - Bgl II
  - Sph I
  - Cla I
  - Sac I
  - Mec+

- **M1-CL1**
  - Bgl II
  - Sph I
  - Cla I
  - Mec+

- **M1-CL1-BGab**
  - Bgl II
  - Sph I
  - Cla I
  - Mec+
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
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

![Nucleotide sequence of the region containing Mec+ gene of S. kasugaensis.](image)

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.
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*.

**References**


4) **KUSUHARA, H. & M. OKANISHI**: in preparation


