Short Communication

THE NUCLEOTIDE SEQUENCE OF THE CHROMOSOMAL TETRACYCLINE-RESISTANCE GENE OF *BACILLUS* *SUBTILIS* R DIFFERS FROM THAT OF *B. SUBTILIS* 168 BY FIVE BASE PAIRS

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We have previously cloned in *Escherichia coli* a 4.5-kbp *Hind*III DNA fragment carrying tetracycline-resistance (Tc^r) determinant (named *tet*BS908) from the *Bacillus subtilis* GSY908 (168 strain) chromosome (8) using plasmid vector pLS353 (5). *tet*BS908 consisted of 1,347 bp encoding 458 amino-acid residues initiating from a GTG codon preceded by a ribosome-binding site (RBS-2). Upstream from *tet*BS908 there exists a short open reading frame (leader peptide) consisting of 20 amino acids initiating from an ATG codon preceded by its own RBS (RBS-1) (7) (see Fig. 1). The 4.5-kbp sequence carrying *tet*BS908 was localized to the replication origin region of the chromosome (1). *B. subtilis* 168 derivatives RM125, 1012 and KS162 also carry the 4.5-kbp *Hind*III sequence (1). The nucleotide sequence (3,070 bp) of the *tet* gene and flanking regions of the 4.5-kbp fragment of the 1012 chromosome agreed perfectly with those of the GSY908 (1).

Southern-blot analysis of the *Hind*III-digested chromosomal DNAs of *B. subtilis* W23, *B. subtilis* R, two *B. subtilis* var. *natto* strains and six other *Bacillus* strains using the 4.5-kbp *Hind*III fragment of the GSY908 chromosome as a probe revealed that only *B. subtilis* R chromosome carries a 3.6-kbp *Hind*III sequence that can hybridize to the probe (1). An attempt has been made in the present study to determine the nucleotide sequence of the 3.6-kbp *Hind*III fragment and compare it with that of the GSY908 chromosome.

Cloning and sequencing of the 3.6-kbp *Hind*III fragment were performed according to the methods previously reported (7,8). The results showed that the

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3.6-kbp fragment actually carries a Tc^r region consisting of promoter, leader sequence encoding 20 amino acids, and Tc^r structural gene (named *tetBSR*), being identical to that of the 4.5-kbp fragment of the GSY908 chromosome except for only five bases within the *tet* gene (Fig. 1). These differences resulted in two amino-acid replacements: an isoleucine at position 439 and a glycine at position 458 in the TET protein of GSY908 were replaced by a threonine (439) and an arginine (458) in that of R. The nucleotide sequences (373 bp) upstream from the *tet*

Fig. 2. Rotation field gel electrophoretic separation of the *AscI*-digested chromosomes of *B. subtilis* R (lane 1) and *B. subtilis* GSY908 (lane 2) and Southern-blot analysis with digoxigenin-labeled probes of the 4.5-kbp *HindIII* fragment of GSY908 chromosome (lanes 1', 2') (panel A) and the 2.1-kbp *SphI-HindIII* fragment of plasmid pAK1 (lanes 1', 2') (panel B).

The set of numbers on the left side in both panels are the molecular size (kbp) of phage λ oligomer DNA series.

promoter — 10 consensus sequence were the same between R and GSY908, while the nucleotide sequences (275 bp) including a putative transcriptional terminator downstream from the TET termination codon were not identical but highly conserved between the two (Fig. 1). Downstream from the conserved sequences only a partial sequence homology were observed.

B. subtilis RM125 competent cells were transformed with pLS353 carrying the 3.6-kbp *HindIII* fragment with *tetBSR*. Tc^r transformants maintaining the plasmid were tested for the level of Tc^r . Maximum Tc concentrations permitting growth of the transformants were determined to be $80\mu\text{g}$ per ml, being the same as that of the transformants of RM125 maintaining pLS353 carrying the 4.5-kbp *HindIII* fragment with *tetBS908* (8). *B. subtilis* RM125 carrying a single copy of *tet* gene could not grow at Tc concentrations above $10\mu\text{g}$ per ml. These results suggest that the above-mentioned two amino-acid replacements of the TET protein do not affect its function.

To analyze physical location of the 3.6-kbp *tetBSR* sequence on *B. subtilis* R chromosome, the *AscI*-digested chromosome of *B. subtilis* R was separated by rotation field gel electrophoresis and hybridized with digoxigenin-labeled probes of

Fig. 3. Genetic and physical maps of the replication origin and Tc^r region of *B. subtilis* 168 chromosome.

DNA fragments used in this study for Southern hybridization probes are shown by stippled boxes in parts *a* and *f*. Part *a*: restriction sites and genetic loci in the origin region (4). Part *b*: partial genetic map of *B. subtilis* 168 chromosome ($11.57 \text{ kbp} = 1^\circ$) (6). Part *c*: 137-kbp *AscI* fragment of *B. subtilis* GSY908 chromosome which carries replication origin region and *tetBS908* (see parts *a* and *f* of the figure and Fig. 2). Part *d*: 133-kbp *AscI* fragment of *B. subtilis* R chromosome which carries replication origin region and *tetBSR* (see parts *a* and *f* of the figure and Fig. 2). Part *e*: partial physical map of *B. subtilis* 168 chromosome. Part *f*: the 12-kbp *EcoRI* fragment of GSY908 chromosomal DNA containing the *tetBS908* and the 10-kbp *EcoRI* fragment of R chromosomal DNA containing the *tetBSR*. Abbreviations used for relevant restriction enzyme sites: E, *EcoRI*; H, *HindIII*; S, *SphI*.

4.5-kbp *HindIII* fragment of the GSY908 chromosome (see part *f* of Fig. 3) and 2.1-kbp *SphI-HindIII* fragment of plasmid pAK1 (3) containing a replication origin region of *B. subtilis* 168 chromosome (see part *a* of Fig. 3). In parallel, the *AscI*-digested chromosome of GSY908 was also analyzed. As shown in Fig. 2(A) and 2(B), both probes gave a discrete hybridization band at 133-kbp *AscI* fragment

in the R chromosome and 137-kbp *AscI* fragment in the GSY908 chromosome. These results indicate that the *tet* gene and the replication origin region of the GSY 908 chromosome are present in the 137-kbp *AscI* fragment and those of the R chromosome in the 133-kbp *AscI* fragment.

The physical map of the *B. subtilis* 168 chromosome has been constructed by Itaya and Tanaka (2); restriction endonuclease cleavage sites and the positions of several genetic markers are mapped in a kilobase pair with the distance from the *SfiI* site (0 kbp) between the 133-kbp *SfiI* fragment and the 238-kbp *SfiI* fragment (see part *e* of Fig. 3). Genetic map is usually shown in a degree (°). One degree (1°) is calculated to be 11.57 kbp. We have previously indicated that in the GSY 908 chromosome the *tet* gene is contained in the 238-kbp *SfiI* fragment and the 12-kbp *EcoRI* fragment possesses a single *SfiI* site (1) (see parts *e* and *f* of Fig. 3). These show that *SfiI* site present in the 12-kbp *EcoRI* fragment corresponds to the *SfiI* site at position 0 kbp. On the basis of the map location of the *abrB* marker at 73 kbp or 3° (2), the *tetBS908* is mapped at around 357° or 5 kbp. The 10-kbp *EcoRI* fragment of the R chromosome containing the *tetBSR* possesses a single *SfiI* site. The region surrounding the *SfiI* site and the upstream region of the *tetBSR* show almost the same restriction endonuclease cleavage pattern as those of the 12-kbp *EcoRI* fragment of the GSY908 chromosome (see part *f* of Fig. 3). These data, taken together, localize the *tetBSR* also at a site close to the origin of replication.

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