Short Communication

A PRECISE DELETION OF *ESCHERICHIA COLI* TRANSPOSABLE ELEMENT *IS1* FROM A PLASMID DURING A COMPETENT CELL TRANSFORMATION IN *BACILLUS SUBTILIS*

HITOSHI AMANO, HIROFUMI ENDO,[†] AND KAZUO SHISHIDO*

*Department of Life Science, Tokyo Institute of Technology,
Midori-ku, Yokohama 227, Japan*

(Received August 1, 1994; Accepted September 13, 1994)

Sakaguchi and Shishido (6) cloned in *Escherichia coli* a 4.5-kbp *Hind*III DNA fragment with the tetracycline-resistance (Tc^r) determinant (named *tet*BS908) from *Bacillus subtilis* GSY908 chromosome using a shuttle plasmid vector pLS353 (4). In this isolation, a 5.2-kbp *Hind*III fragment was also obtained, which was an insertion derivative of the 4.5-kbp fragment (1). It carried *E. coli* transposable element *IS1* (8) flanked by 8-bp duplications of the target sequence, AAAATTTG, just upstream from *tet*BS908 (Figs. 1 and 2) (1). The plasmid pLS353 with the 5.2-kbp *Hind*III fragment was named pTBS1 (Fig. 1) (1, 6). During a competent cell transformation of *B. subtilis*, *IS1* was found to be deleted from pTBS1 (see Figs. 1 and 2) with a high frequency.

Two types of Tc^r transformants were obtained by the competent cell transformation of *B. subtilis* RM125 (9), which maintain either the pTBS1 parent or particular deletion derivative. Nucleotide sequence analysis revealed that the deletion derivative lacks the *IS1* element and one of the 8-bp target sequences for *IS1* insertion (1) (Fig. 2). No other deletion occurred in the plasmid. We examined whether the *IS1* element deleted from pTBS1 had transposed into host chromosomal DNA. The Tc^r transformant was treated with ethidium bromide to obtain plasmidless Tc^s derivatives. The chromosomal DNAs were then isolated from the Tc^s derivatives, digested with *Hind*III, and subjected to Southern blot hybridization (7) with a digoxigenin-labeled probe (the 0.6-kbp *Pvu*II-*Tth*III fragment of *IS1* element; part (a) of Fig. 2). No hybridization signal was obtained

* Address reprint requests to: Dr. Kazuo Shishido, Department of Life Science, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 227, Japan.

[†] Present address: Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Machida 194, Japan.

Fig. 1. Structure of pTBS1.

Vector pLS353 (open region), the *tetBS908*-carrying *Bacillus subtilis* chromosomal DNA segment (stippled regions), and IS1 (closed region) are shown. IS1-encoded genes *insA* and *insB* (5) are indicated as open arrows and antibiotic-resistance genes are indicated as solid arrows.

Fig. 2. Simplified structures of IS1 in pTBS1 and IS1 mutant in pTBS1mm (a) and nucleotide sequence after deletion of IS1 and IS1 mutant in the competent cell transformations of *Bacillus subtilis* (b).

Plasmid pTBS1mm carries a defective IS1 element which lacks the nucleotides 234–435 of IS1. *ins* genes and its derivatives are indicated as open arrows with stop codons. The 8-bp target sequences for IS1 insertion are boxed.

for 10 samples tested (data not shown), showing an absence of IS1 element in their chromosomes.

We investigated the correlation between competence and frequency of IS1 deletion. As shown in Fig. 3, the competence of *B. subtilis* RM125 was associated with an early stationary phase of growth in the first stage culture. It was highest at 3.3 h at which the frequency of IS1 deletion was maximum (~80%). At 2.5 h and around 4 h, the frequency of IS1 deletion was 50–60%. The results show that the frequency of IS1 deletion coincides with the competence of *B. subtilis*.

Fig. 3. Correlation between competence and *IS1* deletion during the competent cell transformation of *Bacillus subtilis* with the *IS1*-carrying plasmid pTBS1.

Recipient RM125 cells were grown at 37°C in a glucose minimal medium supplemented with a mixture of amino acids for first stage culture (2). After the indicated times, $\sim 10^8$ cells were withdrawn and inoculated into the fresh minimal medium (10 ml) for second stage culture (2). After incubation for 2 h at 37°C, competent cell suspensions were used for transformation with pTBS1. Cell density (open circles), competence (open squares) and frequency of *IS1* deletion (open triangles) are shown. Levels of competence are shown as the numbers relative to the maximum observed at 3.3 h of the first-stage culture.

To examine whether the entire *IS1* is necessary for its deletion, a modified plasmid pTBS1mm was constructed which lacked nucleotides 234–435 of *IS1* (part (a) of Fig. 2). For construction of this plasmid, the *Mlu*I-linearized pTBS1 was blunted by treatment with mung bean nuclease and then digested with *Msc*I. The resulting 11.0-kb fragment was recircularized with T4 DNA ligase. The deletion of nucleotides creates a stop codon TGA in the open reading frame *insA* reported by Ohtsubo and Ohtsubo (5). pTBS1mm (and pTBS1) were introduced into RM125 competent cells prepared at the 4-h culture according to the method developed by Anagnostopoulus and Spizizen (2). Tc^r transformants (24 each) isolated were analyzed for the frequency of *IS1* deletion. As shown in Table 1, pTBS1mm exhibited a deletion frequency of 42%, being similar to that of pTBS1 (54%). The results show that the entire *IS1* sequence is not necessary for its deletion.

We introduced pTBS1 into protoplasts of *B. subtilis* RM125, but all 96 Tc^r colonies tested maintained pTBS1, suggesting that an uptake of the *IS1*-carrying plasmid into *B. subtilis* competent cells is necessary for deletion of *IS1* sequence.

Table 1. Frequency of deletion of *IS1* sequence.

Plasmid introduced	Reduced size (bp) in <i>IS1</i>	Plasmid carried in 24 transformants	
		Parental	Deleted
pTBS1	0	11	13
pTBS1mm	202	14	10

Conditions of the competent cell transformation are shown in the legend of Fig. 2. The first stage culture was done for 4 h.

Based on the model of DNA processing during plasmid transformation of *B. subtilis*-competent cells presented by Canosi et al. (3), the mechanism of the *IS1* deletion may be explained as follows (Fig. 4). The single-stranded form of pTBS1 DNA pairs with a complementary sequence of the host *B. subtilis* chromosome through the function of the RecE4 protein, etc. The *IS1* segment and one of the 8-bp target sequences which cannot pair with the chromosome is looped out. This type of DNA interaction is not expected to occur in a protoplast transformation. The pair of single-stranded pTBS1 and *B. subtilis* chromosomal DNA sequence is cut by a nuclease at junction sites (shown by arrows in the figure) and the liberated heteroduplex DNA is filled by DNA polymerase to double-stranded circular form. Parental pTBS1 and its *IS1*-deleted plasmid are generated by replication of the

Fig. 4. Schematic representation of the mechanism of *IS1* deletion.

The 4.5-kbp *Hind*III fragment (solid thick line) of *Bacillus subtilis* chromosomal DNA (dotted thick line), plasmid vector pLS353 sequence (solid thin line), and *IS1* element (dotted thin line) are shown.

repaired plasmid, and then either plasmid is lost through incompatibility. When complementary single-stranded pTBS1 DNA molecules anneal to themselves, no *IS1*-deleted plasmid is generated. This model, however, does not completely explain the fact that the *IS1*-deletion plasmid was more frequently seen in the *B. subtilis* host with higher competence (Fig. 3). Probably *B. subtilis* cells have an unknown system of excision of transposable elements, and the activity of this system is correlated with competence of the cells. To verify this idea, a more detailed analysis is necessary.

REFERENCES

- 1) Amano, H., Sakaguchi, R., and Shishido, K., An insertion of *Escherichia coli* transposable element *ISIK* into the site immediately before tetracycline-resistance determinant of *Bacillus subtilis* chromosomal DNA fragment in cloning in *E. coli*. *FEMS Microbiol. Lett.*, **79**, 5–8 (1991).
- 2) Anagnostopoulos, C. and Spizizen, J., Requirement for transformation in *Bacillus subtilis*. *J. Bacteriol.*, **81**, 741–746 (1961).
- 3) Canosi, U., Iglesias, A., and Trautner, T. A., Plasmid transformation in *Bacillus subtilis*: Effects of insertion of *Bacillus subtilis* DNA into plasmid pC194. *Mol. Gen. Genet.*, **181**, 434–440 (1981).
- 4) Ogawa, H., Sekiguchi, T., Shishido, K., and Nosoh, Y., Molecular cloning of promoter-containing fragments from *Bacillus stearothermophilus* and their expression in *Escherichia coli* and *Bacillus subtilis*. *FEMS Microbiol. Lett.*, **24**, 168–172 (1984).
- 5) Ohtsubo, H. and Ohtsubo, E., Nucleotide sequence of an insertion element, *IS1*. *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 615–619 (1978).
- 6) Sakaguchi, R. and Shishido, K., Molecular cloning of tetracycline-resistance determinant from *Bacillus subtilis* chromosomal DNA its expression in *Escherichia coli* and *B. subtilis*. *Biochim. Biophys. Acta*, **949**, 49–57 (1988).
- 7) Southern, E. M., Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*, **98**, 503–517 (1975).
- 8) Umeda, M. and Ohtsubo, E., Mapping of insertion elements *IS1*, *IS2* and *IS3* on the *Escherichia coli* K-12 chromosome. Role of the insertion elements in formation of Hfrs and F' factors and in rearrangement of bacterial chromosomes. *J. Mol. Biol.*, **208**, 601–614 (1989).
- 9) Uozumi, T., Hoshino, T., Miwa, K., Horinouchi, S., Beppu, T., and Arima, K., Restriction and modification in *Bacillus subtilis* species. Genetic transformation of bacteria with DNA from different species. *Mol. Gen. Genet.*, **152**, 65–69 (1977).