**StyD41 Restriction-Modification System of Salmonella Typhi D4: Cloning and Sequence Analysis**

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A plasmid (5.4 kbp) from *Salmonella Typhi* D4 has been identified as encoding a restriction and modification (R-M) system. DNA fragments (2537 bp) that carried the genes for restriction endonuclease and methyltransferase encoded on the plasmid were sequenced. Two divergently arranged open reading frames of 957 bp for the restriction endonuclease consisting of 318 aa (amino acids) and 1140 bp for the DNA methyltransferase consisting of 379 aa were identified. These sequences were similar to the sequences of the *SsoI R-M* system, including the interspace between the two genes.

**Key words** Restriction-modification system; sequence analysis; StyD41; pSTD4; *Salmonella* Typhi D4; deletion mutant

Previously, we reported on *StyD4* restriction endonuclease (ENase) of *Salmonella Typhi* D4 (S. Typhi D4).1–2 S. Typhi D4 has a plasmid, named pSTD4 (5.4 kbp), which encodes ENase recognizing the CCNGG DNA sequence and the related modification methyltransferase (MTase). The methylase protect DNA against the attack of *StyD4* ENase. The *StyD4* methylase is classified as a DNA (cytosine-5) methyltransferase (m5C-MTase). The m5C-MTases methylate the 5-carbon of the pyrimidine ring of cytosine. They share a large set of well-conserved blocks of amino acid sequences, which are called motifs.3 Many isochizomers have been reported.4 Of these, DsaV, BsoXI and SsoII were reported to have the same cleavage site as *StyD4*. The cleavage site of *StyD4* had | CCNGG the same as DsaV5 and SsoII,6 but not the same as the CC | NGG of ScrFI.7 For comparison with the sequences of other isochizomers, the cloning and sequence analysis of the *StyD4* restriction-modification (R-M) system have been carried out.

**MATERIALS AND METHODS**

Previously, a restriction map of pSTD4 (5400 base pairs (bp)) was reported.2 According to the map, EcoRI fragments were selected for cloning. We obtained five kinds of EcoRI fragments. These fragments of pSTD4 were inserted into EcoRI-digested dephosphorylated pUC118, with T4 DNA ligase. The ligated DNA was used for the transformation of *E. coli* WA803, and ampicillin-resistant (Ap+) transformants were obtained. pUC118 has twelve *StyD4* sites. We tested the activity of ENase and MTase. The activity of ENase was measured using the methods described before.1 Also, a cross-streak method (as follows) was used as a highly sensitive method. On a broth plate, a phage solution (x cts 857 or T3) was painted in a straight belt. Across the phage belt, bacteria solution was also painted. After culture overnight, bacteria having ENase grew but bacteria without ENase did not at the site across the phage belt. MTase activity was indirectly detected. After isolation of the plasmid, each plasmid was cleaved by *StyD4*.

Plasmid which resisted cleavage by *StyD4* was estimated to encode MTase. Plasmid DNA was isolated by the alkaline lysis method.8 The location of ENase and MTase genes on pSTD4 was estimated.

Deletion mutants were made by the Deletion Kit for Kilo-sequencing (Takara). The cleaving of a BamHI-PstI fragment from the starting DNA started the deletion reaction. Sequence deletion was constructed by Exonuclease III according to the manufacturer’s instruction. Many mutants, with different sized plasmid, were tested for their activity of ENase and MTase. *E. coli* WA803 was used as the host. These deletion mutants were also used for sequencing.

Insertion mutants were used for the search for the ENase gene on pSTD4. pKDEC100 (pHSG415CM::Tn3)9,10 was used as a transposon-carrying and temperature-sensitive plasmid. Competent cells of WA803 carrying pSTD4 were transformed by the plasmid. These transformants were cultured on the ampicillin (100 μg/ml) and chloramphenicol (25 μg/ml)-containing plates at 30°C overnight. After this selection, further culture was accomplished on the ampicillin-containing (at the first time 200 and at the second 100 μg/ml) plate at 42°C overnight. Transposon Tn3 has an ampicillin resistant gene. By these treatments, cells having a Tn3 inserted genome or plasmid can survive on the ampicillin-containing plate.

The molecular weight of ENase, *StyD4* was measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and gel filtration on a Superox 12 (Pharmacia, HR 10/30, FPLC).

For the sequencing work, *E. coli* MV1184 was used with a helper phage, M13K07. Single DNA was obtained from a transformant of MV1184. Of all the sequences of pSTD4, 2537 sequences were determined by the dideoxynucleotide chain-termination method.11

Search homology was accomplished by GENETYX-MAC (Software Development Co., Ltd., Tokyo).

**RESULTS AND DISCUSSION**

EcoRI fragment-inserted clones lost their ENase activities. The ENase gene was recognized to be over the

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Fig. 1. ENase and MTase Activities of Deletion Derivatives

Activities were assayed by the methods described in Materials and Methods. +, detectable level; -, no detectable activity. The ligation site was shown as a line in the figure of the clone (5400-1 and 5400-2). The big column shows the estimated location of the MTase gene on pSTd4.

Fig. 2. ENase and MTase Activities of Insertion Mutants of pSTd4

Small arrows show the Tn3-inserted site on pSTd4. The big column shows the estimated location of the ENase gene on pSTd4 and the sequencing area.

EcoRI site of pSTd4. MTase activities remained on the 1—1736 bp position on the clone (5400-1 etc.), as shown in Fig. 1. Analysis of the insertion of mutants also showed these data (Fig. 2). Three insertion mutants (which showed negative activities of ENase) were obtained. The ENase gene covers the EcoRI sites (2 and 3). The gene area for the StyD41 R-M system seemed to be in the 2537 bp of pSTd4. After sequencing, the 2537 bp area was searched for its amino acid sequences. Two divergently arranged open reading frames were found, as shown in Fig. 3. These DNA and amino acid sequence data (StyD41 R-M system) have been deposited in a GenBank database under accession No. D73442. By the gel filtration method, the molecular weight (MW) of StyD41 was estimated to be 70000 Da. On the other hand, on SDS-PAGE we estimated the MW to be 35000 Da. From these data, we concluded that StyD41 might act as a dimer and the MW of StyD41 (its monomer MW weight) might be 35000 Da. These data coincided with the MW of ENase calculated on open reading frames.

The 2537 bp DNA sequences show 98.6% homology to the area (2648 bp) for the gene of the SsoII R-M
The deduced amino acid sequences of the StyD41 R-M system were analyzed. All ten motifs were also conserved in the DNA sequence of StylD41 MTase. The MTases of StylD41, DsaV, and ScrFI are all m5C-MTases and share such motifs. Both the deduced amino acid sequences in all ten motifs of StyD41 and StylD41 MTase were same. At the Motif I and X site, DNA sequences of several m5C-MTases were compared with each other (data not shown). As a result, it is recognized that the DNA sequences in these motifs are also conserved to some extent.

REFERENCES AND NOTES


Fig. 3. Nucleotide Sequences of the ENase and MTase Gene

The deduced aa sequences for ENase and MTase are shown below or above the gene sequences, respectively. U shows a stop codon in the amino acid sequences.