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Absence in *Bacillus subtilis* and *Staphylococcus aureus* of the Sequence-Specific Deoxyribonucleic Acid Methylation That Is Conferred in *Escherichia coli* K-12 by the *dam* and *dcm* Enzymes

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Restriction analysis of plasmid pHV14 deoxyribonucleic acid isolated from *Escherichia coli* K-12, *Bacillus subtilis*, and *Staphylococcus aureus* with restriction endonucleases *Mbo*I, *Sau*3AI, and *Eco*RII was used to study the methylation of those nucleotide sequences which in *E. coli* contain the major portions of N^6-methyladenine and 5-methylcytosine. The results showed that neither *B. subtilis* nor *S. aureus* methylates deoxyribonucleic acid at the same sites and nucleotides which are recognized and methylated by *dam* and *dcm* enzymes in *E. coli* K-12.

Two sequence-specific DNA methylases have been observed in *Escherichia coli* K-12 (14, 18) besides the enzyme involved in host-specific DNA modification. One of the two enzymes is coded by the *dam* gene and converts the adenine residue in the sequence GATC to N^6-methyladenine (7, 13, 17). The other is specified by the *dcm* gene and methylates the internal cytosine residue in the sequence CC(A/T)GG to 5-methylcytosine (24). The existence of two methylases of similar specificity has been demonstrated in two bacterial strains closely related to *E. coli*, *Salmonella typhi* and *Salmonella typhimurium* (10, 15).

An important biological role has been ascribed to the *dam* methylation in the course of post-replicative repair of nucleotide misincorporations produced during DNA synthesis. Experimental data obtained with mutants defective in the *dam* methylase suggest that in the wild type the delayed methylation of DNA after replication allows repair enzymes to discriminate between parental and newly synthesized DNA strands in order to conserve the parental information during mismatch repair (9). As yet no specific function has been attributed to the *dcm* methylation. However, it has been assumed that *dam*- or *dcm*-methylated bases or both may also interfere with the stability of plasmids in bacteria (10, 17). Some arguments and data have also been provided implying that *dam* and *dcm* methylation affect initiation of replication (21) and strand elongation (11).

Site-specific methylation of DNA can be studied by restriction analysis using pairs of isoschizomeric restriction endonucleases differing in cleavage specificity with respect to methylated bases in their recognition sites. This sensitive approach bas been applied repeatedly (7, 10, 17, 21). However, the fact that the state of site-specific DNA methylation may affect the cleavage of the DNA by several restriction endonucleases with identical or overlapping recognition sites poses an experimental problem as to the choice of the appropriate endonucleases for restriction analysis or molecular cloning experiments with a given DNA. To avoid erroneous results one has to consider the methylation of DNA from an organism (2). In this view it seemed desirable to compare the methylation pattern of DNA from *E. coli* K-12 with that of *Bacillus subtilis* and *Staphylococcus aureus* since in both of these gram-positive bacteria molecular cloning has recently been achieved (8, 12, 16, 23). In addition we were interested in determining whether enzymes equivalent in specificity to the *dam* and *dcm* methylases of *E. coli* K-12 are also present in *B. subtilis* and *S. aureus*.

To compare the methylation of the sequences GATC and CC(A/T)GG in the DNA from various organisms, we used the DNA from a plasmid which can replicate in *E. coli*, *B. subtilis*, and *S. aureus*. Such a plasmid is pHV14 (8), which consists of the *E. coli* plasmid pBR322 (3) and the *S. aureus* plasmid pC194 (12). This plasmid was transformed into *E. coli* K-12 strains C600 (*dam*$^+$ *dcm*$^+$) and GM119 (*dam-3 dcm-6*; 18) by using Ca^{2+}-treated cells (5), and into *B. subtilis* (strain Marburg 168) and *S. aureus* W57 (23) by the spheroplast-polyethyleneglycol method (4, 23). pHV14 DNA was isolated from *E. coli* with Triton X-100 lysates (7), from *B. subtilis* with sodium dodecyl sulfate-sodium chloride lysates

(19), and from *S. aureus* by the acetone-spheroplast method (20).

Adenine methylation of the sequence GATC was monitored by restriction with isoschizomeric enzymes *Mbo*I and *Sau*3AI. *Mbo*I cleaves at the sequence only when the sequence is nonmethylated, whereas *Sau*3AI cleaves it irrespective of methylation (7). The results of restriction analysis of the various pHV14 DNA preparations with *Mbo*I and *Sau*3AI are shown in Fig. 1. Plasmid DNA from *E. coli dam*$^+$ was completely cleaved by *Sau*3AI but not by *Mbo*I (Fig. 1, lanes b and a), indicating that virtually all GATC sequences were methylated by the *dam* methylase. After replication in *E. coli dam-3* the pHV14 DNA was no longer refractory to *Mbo*I, resulting in a fragment pattern indistinguishable from that obtained with *Sau*3AI (Fig. 1, lanes c and d). On the other hand, plasmid DNA from *B. subtilis* and *S. aureus* gave restriction patterns identical for *Mbo*I and *Sau*3AI and also identical to that obtained with DNA from the *dam* mutant of *E. coli* K-12 (Fig. 1, lanes e, f, g, and h). This demonstrates that adenine methylation of GATC sequences in the DNA of both *B. subtilis* and *S. aureus* is absent.

Comparison of the *Sau*3AI fragment patterns of pHV14 and pBR322 indicated the presence of only two GATC sites in that 1.8-megadalton part of the composite plasmid which represents the *S. aureus* plasmid pC194 (data not shown). Thus, the frequency of GATC sites in pC194 is less than 1/5 that expected on a random basis.

To determine the extent of cytosine methylation in CC($\overset{A}{T}$)GG sequences, we employed *Eco*RII, which cleaves this site only when not methylated at the internal C either by the *dcm* methylase or the *Eco*RII modification enzyme. As shown in Fig. 2 (lane a), the pHV14 DNA from *E. coli dcm*$^+$ was resistant to *Eco*RII, since the same band pattern was observed with DNA from a control incubation which did not contain *Eco*RII (not shown). On the other hand, plasmid DNA from an *E. coli* K-12 mutant deficient in the *dcm* methylase was completely cleaved into distinct fragments (Fig. 2, lane b). Plasmid DNA isolated from *B. subtilis* and *S. aureus* was also cleaved by *Eco*RII into fragments identical to those obtained from the *dcm* mutant DNA, indicating that DNA from the two gram-positive strains does not contain 5-methylcytosine at positions equivalent to those observed in DNA of *E. coli dcm*$^+$ (Fig. 2, lanes c and d).

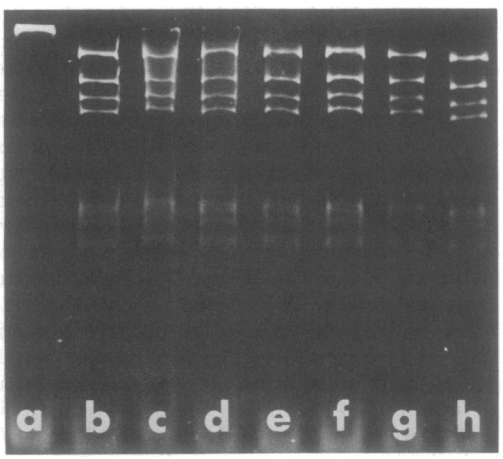

FIG. 1. *Acrylamide gel electrophoresis of pHV14 DNA from various strains after treatment with restriction endonucleases MboI or Sau3AI. DNA from: (a) E. coli dam*$^+$ *plus MboI; (b) E. coli dam*$^+$ *plus Sau3AI; (c) E. coli dam plus MboI; (d) E. coli dam plus Sau3AI; (e) B. subtilis plus MboI; (f) B. subtilis plus Sau3AI; (g) S. aureus plus MboI; (h) S. aureus plus Sau3AI. Acrylamide concentration was 6%. The electrophoresis (gel size 0.2 by 16 by 11 cm) was run at 50 mA for 4 h as described (7). MboI was purchased from Biolabs (Beverly, Mass.); Sau3AI was isolated by the method of Sussenbach et al. (25). The reaction conditions for cleavage by MboI and Sau3AI were: 6 mM Tris-hydrochloride (pH 7.5), 60 mM NaCl, 15 mM* $MgCl_2$. *About 2 μg of plasmid DNA was incubated with about 2 U of MboI or Sau3AI for 1 h in a volume of 50 μl. The incubation temperature was 37°C for MboI and 30°C for Sau3AI.*

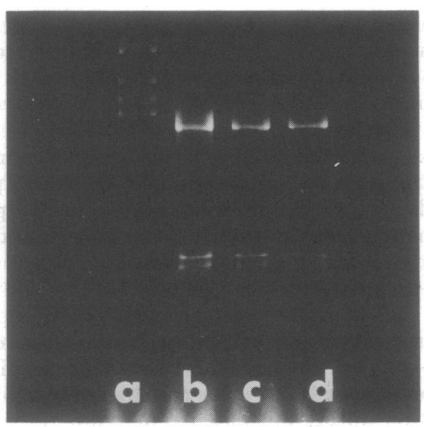

FIG. 2. *Agarose gel electrophoresis of pHV14 DNA from various strains after treatment with restriction endonuclease EcoRII. DNA from: (a) E. coli dcm*$^+$*; (b) E. coli dcm; (c) B. subtilis; (d) S. aureus. Agarose concentration was 1.3%. The electrophoresis (gel size 0.5 by 16 by 11 cm) was run at 50 mA for 2.5 h as described (7). EcoRII was isolated from E. coli RY22 by the method of Roulland-Dussoix et al. (22). The reaction conditions for cleavage by EcoRII were: 15 mM Tris-hydrochloride (pH 8.2), 10 mM* $MgCl_2$, *60 mM KCl, and 15 mM β-mercaptoethanol. About 1 μg of plasmid DNA was incubated with EcoRII overnight at room temperature.*

The results show that the sequence-specific methylation of adenine and cytosine that is produced in *E. coli* K-12 by *dam* and *dcm* enzymes does not occur in *B. subtilis* 168 and *S. aureus* W57. The *dcm* methylase does not appear to have an important function in all *E. coli* strains, since wild-type *E. coli* B does not have this activity (1, 26). On the other hand, N^6-methyladenine and 5-methylcytosine residues have been detected in the DNA of *B. subtilis* 168 (6), and N^6-methyladenine has been detected in the DNA of *S. aureus* (26). Thus, it is possible that enzymes of different specificity but similar function to the *dam* and *dcm* methylases may exist in gram-positive bacteria.

Our results also show that several restriction endonucleases which are inactive on plasmid DNA from *E. coli* K-12 due to sequence-specific DNA methylation may be used for restriction analysis of DNA from both *B. subtilis* 168 and *S. aureus* W57. These restriction enzymes having recognition sites identical to or including the *dam* and *dcm* sites are *Mbo*I, *Dpn*II (GATC), *Bcl*I (TGATCA), and *Eco*RII [CC($\frac{A}{T}$)GG].

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