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DNA Adenine Methylation of *sams1* Gene in Symbiont-Bearing *Amoeba proteus*

Taeck J. Jeon

Department of Biology, College of Natural Sciences, Chosun University, Gwangju 501-759, Republic of Korea

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The expression of amoeba *sams* genes is switched from *sams1* to *sams2* when amoebae are infected with *Legionella jeonii*. To elucidate the mechanism for the inactivation of host *sams1* gene by endosymbiotic bacteria, methylation states of the *sams1* gene of D and xD amoebae was compared in this study. The *sams1* gene of amoebae was methylated at an internal adenine residue of GATC site in symbiont-bearing xD amoebae but not in symbiont-free D amoebae, suggesting that the modification might have caused the inactivation of *sams1* in xD amoebae. The *sams1* gene of xD amoebae was inactivated at the transcriptional level. Analysis of DNA showed that adenine residues in *L. jeonii sams* were also methylated, implying that *L. jeonii* bacteria belong to a Dam methylase-positive strain. In addition, both SAM and Met appeared to act as negative regulators for the expression of *sams1* whereas the expression of *sams2* was not affected in amoebae.

***Keywords*:** endosymbiosis, S-adenosylmethionine, gene regulation, symbiogenesis

The xD strain of *Amoeba proteus* arose from the D strain by spontaneous infection with *Legionella jeonii* (Originally called X-bacteria; Jeon and Lorch, 1967), and xD amoebae are now dependent on their symbionts for survival. The *L. jeonii* bacteria were phylogenetically identified based on the analyses of bacterial 16S rRNA, *rpoB*, and *mip* genes (Park *et al*., 2004). As a result of harboring *L. jeonii* (about 42,000 symbionts in each xD amoeba), xD amoebae exhibit various physiological characteristics that are different from those of symbiont-free D amoebae (Jeon, 2004). One of them is that symbiont-bearing xD amoebae do not transcribe the *sams1* gene encoding SAMS1 and hence no longer produce their own SAMS1 enzyme (Choi *et al*., 1997; Jeon and Jeon, 2003; Jeon and Jeon, 2004).

S-Adenosylmethionine synthetase (SAMS) is an essential cellular enzyme that catalyzes the formation of S-adenosylmethionine (SAM) from methionine (Met) and ATP (Thomas and Surdin-Kerjan, 1997; Mato *et al*., 2002; Mato and Lu, 2007). *Amoeba proteus* contains two *sams* genes, *sams1* and *sams2*. The expression of the genes is switched from *sams1* to *sams2* when amoebae are infected with *L. jeonii*, and it has been suggested that the switch in the expression of host *sams* genes caused by bacteria might play a role in the development of symbiosis and the host-pathogen interactions (Jeon, 2004; Jeon and Jeon, 2004). However, the mechanism for the switch in the expression of host *sams* genes by endosymbiotic bacteria is unknown.

The genomic DNA of most organisms is modified by methylation at the C-5 or N-4 position of cytosine and at the N-6 position of adenine (Low *et al*., 2001; Vanyushin, 2006; Wion and Casadesus, 2006). Methylation of DNA plays a role in a variety of biological processes such as regulation of gene expression, DNA replication, mismatch repair, host-pathogen interactions, and in cellular defense against foreign DNA (Low *et al*., 2001; Bhagwat and Lieb, 2002; Oshima *et al*., 2002). Methylation of cytosine residues in CpG sites is a common feature in eukaryotes (Lee, 2003; Vanyushin, 2006), while adenine and/or cytosine are methylated, depending on the species, in prokaryotes (Wion and Casadesus, 2006; Heusipp *et al*., 2007). The DNA adenine methyltransferase (Dam) catalyzes methylation of adenine at the N-6 position in GATC sequences. Dam methylases are found in several strains of Gram-positive and Gram-negative bacteria including *Legionella.* A growing number of reports show the presence of DNA adenine methylation in lower eukaryotes such as *Chlorella* spp., *Chlamydomonas reinhardtii*, and ciliates including *Tetrahymena thermophila*, *Paramecium Aurelia*, *Oxytricha fallax*, and *Stylonichia mytilius* (Hattman, 2005).

In this study, to elucidate the mechanism for the inactivation of host *sams1* gene by endosymbiotic bacteria, methylation states of the *sams1* gene of D and xD amoebae was compared. The results show that some adenine residues of amoeba *sams1* were methylated in symbiont-bearing xD amoebae but not in symbiont-free D amoebae, suggesting that the modification might be responsible for the inactivation of *sams1* in xD amoebae.

Materials and Methods

Amoebae

The D and xD strains of *A. proteus* were cultured in a modified Chalkley's solution (Jeon and Jeon, 1975) in Pyrex baking dishes (35×22×4 cm). Amoebae were fed daily with axenically cultured and washed *Tetrahymena pyriformis* as food organisms (Goldstein and Ko, 1976).

∗ To whom correspondence should be addressed.
(Tel) 82-62-230-6654; (Fax) 82-62-234-4326
(E-mail) tjeon@chosun.ac.kr

Nuclear run-on assay

Nuclei were prepared by a modified method of Choi and Jeon (1989). Amoebae were homogenized, filtered through a 45-μM-pore-size nylon screen, and then centrifuged at successive accelerations of 70, 250, and 800×g. The resuspended pellet in 20-mM Tris buffer containing 0.5% sorbitol was laid on top of a 30/20/10% Percoll gradient. Nuclei were collected from the 20/10% interface after centrifugation for 10 min at 1,000×g.

In vitro transcription using isolated nuclei was carried out as described by Dehio and Schell (1994) with a little modification. The pelleted nuclei (1×10^6) were mixed with 400 μl of reaction buffer [40-mM Tris; pH 8.0, 10-mM $MgCl_2$, 50-mM KCl, 0.1-mM DTT, 25% glycerol, 400 U of Rnasin, 1-mM each of ATP, GTP, CTP, and 200 μCi of [^{32}P]UTP (ICN, 3000 Ci/mmol)] and incubated for 30 min at 30°C with shaking. The reaction was stopped by the addition of 100 units of RNase-free DNase I and 10 μl of 200-mM $CaCl_2$, and then treated with 10 μl of 20 mg/ml Proteinase K. After additional incubation for 30 min at 37°C, the [^{32}P]-labeled RNA was purified and hybridized to Hybond nylon membrane with immobilized spots containing 5 or 10 μg of *sams1*, myosin, or 18S rRNA. The membrane was exposed to X-ray film after hybridization for 2 days at 65°C.

Southern and Western blot analyses

Total DNA was prepared from D and xD amoebae by DNeasy Tissue Kit (QIAGEN). For analysis of DNA adenine methylation, 10 μg of DNA was digested with *Dpn*I, *Dpn*II, and *Sau*3AI. For testing methylation states of cytosine residues in CpG sites, *Bsi*E1, and *Hin*fI were used. Southern-blot analysis was performed by the standard procedure. For probing *sams1* and *L. jeonii sams* genes, 0.9-kb fragments (nt -530 to 349) of *sams1* and 0.7-kb fragments (nt -348 to 398) of *L. jeonii sams* (Jeon and Jeon, 2003) were amplified by PCR and labeled with [^{32}P]-dCTP by the Prime-a-Gene labeling system (Promega).

For Western blot analysis, proteins were extracted from amoebae grown in various concentrations of 5-aza-2'-deoxycytidine (Sigma) for 6 days and immunoblotted with a polyclonal antibody (pAb) against amoeba-SAMS1 (Jeon and Jeon, 2003).

***In vitro* transcription using amoeba nuclear extracts**

In vitro transcription using amoeba nuclear extracts was performed by a modified method of Shapiro *et al*. (1988). As a DNA template in the *in vitro* transcriptions, the *sams1* gene, from nt -530 to 1212, was amplified and cloned into pGEM easy vector (Promega). Methylated *sams1* was prepared by introducing the plasmid into JM109 and unmethylated *sams1* DNA into INV110 (Invitrogen). As a negative control, a 1.1-kb fragment (nt 106∼1212) of *sams1* inserted into the pGEM easy vector was used. As a positive control, pBig-GFPmyo containing actin promoter 15 of *Dictyostelium* (kindly provided by Dr. J. A. Spudich; Moores *et al*., 1996) was used. The actin promoter 15 was isolated by digestion with *Bam*HI and cloned into the pBSKII$^+$ vector.

The RNA was extracted as described in run-on assay and transcripts of *sams1* and actin were detected by primer-extension experiments (Jeon and Jeon, 2003). For detecting transcripts of *sams1*, antisense oligonucleotides corresponding to nt 33∼55 relative to the translation-initiation site of *sams1* were used in the primer-extension experiments. For detecting transcripts of actin and *sams1* genes used as positive and negative controls, a plasmid-specific primer: T7 primer; 5'-TAATACGACTCACTATAGGGCGA-5', and an oligonucleotide primer complementary to nt 139∼158 of the *sams1* gene, respectively, were used.

Bisulfite genomic sequencing

Bisulfite genomic sequencing was done as described by Ghoshal *et al*. (2000). DNA (5 μg) of D and xD amoebae was denatured in 0.3-M NaOH for 30 min at 37°C in 10 μl, mixed with 100 μl of 2-M sodium metabisulphite (Sigma) containing 0.5-mM hydroquinone (pH 5.0) and cycled in a thermal cycler for 30 min at 50°C and for 2 min at 95°C for 20 cycles. Bisulfite-treated DNA was desalted using a Wizard DNA Clean-up Kit (Promega), eluted in 100 μl of H_2O, and DNA was desulfonated in the presence of 0.3-M NaOH for 30 min at 37°C. The solution was neutralized by addition of NaOAc (pH 4.5) to 0.2-M (final concentration).

The bisulfite-converted DNA was desalted again as before, eluted in 70 μl of H_2O, and an aliquot (0.5∼1 μl) was used for subsequent PCR amplification. The promoter region of *sams1* was amplified with two modified primers, SAM1F; 5'-CGAATGTAGAAATCATTGGAG-3' and SAM2R primers; 5'-AAATATCCTCTACTAACTAC-3', corresponding to the nucleotides from nt -530 to -509 and nt 52 to 33 in the *sams1* gene (Jeon and Jeon, 2003), respectively, from the bisulfite-treated DNA of amoebae. To avoid any nonspecific amplification, the products obtained from the first round of PCR were amplified with another set of primers, SAM1F and SAM3R or SAM2R and SAM3F, under the same PCR condition, with the exception that the annealing temperature was maintained at 59°C. The nucleotides of SAM3R primer; 5'-TTTACATCACTACTACAATC-3', were located at nt -219 to -200 of the *sams1* gene, and SAM3F primer at the same position as SAM3R but in reverse sequences. The PCR products were sequenced using the T7 Sequenase Version 2.0 DNA Sequencing Kit (USB) and SAM1F or SAM3F primer.

Reverse transcription-PCR (RT-PCR)

An aliquot (1 μg) of total RNA was reverse-transcribed with MMLV reverse transcriptase (Promega) and Oligo (dT) primers. Transcripts of amoeba *sams* genes were amplified with gene-specific primers. For amplifying *sams1* gene products, a forward primer, located at nt -46 to -25, and a reverse primer, located at nt 935 to 916 were used. In the case of *sams2*, a set of primers was used, located at nt 435 to 452 and at nt 732 to 715. Amoeba's myosin primers (Oh and Jeon, 1998) were used as internal controls.

Results

Inactivation of *sams1* in xD amoebae

To determine if inactivation of *sams1* in xD amoebae occurred at the transcriptional level or post-transcriptional level, run-on assay was performed using intact nuclei isolated from D and xD amoebae (Fig. 1). When D amoeba

nuclei were used in the assay, the transcript of *sams1* was detected but no transcript of *sams1* was detectd when xD amoeba nuclei were used in the assay. The transcripts of *myosin* and 18S rRNA were detected in assays using either D or xD amoeba nuclei. These results indicate that the *sams1* gene in xD amoebae is inactivated at the transcriptional level.

DNA adenine methylation in GATC sites of *sams1* in xD amoebae

To determine if the expression pattern of *sams1* in amoebae is correlated with dam-site methylation status of *sams1* promoter in D and xD amoebae, Southern blot analysis was employed using the methylation-sensitive restriction isoschizomers *Dpn*II and *Sau*3AI (Both enzymes recognize the GATC sequence, but *Dpn*II cannot cleave if the internal A is methylated whereas *Sau*3AI cleaves regardless of the methylation of the recognition sites). Genomic DNA samples from D and xD amoebae were digested with *Dpn*II or *Sau*3AI, and then analyzed by Southern blotting using 0.9-kb fragments (nt -530 to 349) of *sams1* as the probe (Fig. 2A). The *sams1* gene of D amoebae was not methylated at GATC sites as indicated by the presence of one fragment of 520 bp in the *Dpn*II-treated samples, consistent with cleavage by *Sau*3AI. In contrast, while cleavage of xD-amoeba DNA with *Sau*3AI resulted in the same pattern as obtained in D amoebae, *Dpn*II could not cleave with the same efficiency as that in D amoebae and generated higher molecular-mass bands of approximately 2 kb and 1 kb together with a band of similar size found in D amoeba. These results indicate that adenine residues of some recognition sites of *Dpn*II in xD-amoeba *sams1* are partially methylated (Fig. 2A).

To examine the methylation status of the *L. jeonii sams* gene, the genomic DNA of *L. jeonii* was digested with *Dpn*I, *Dpn*II or *Sau*3AI, and analyzed by Southern blotting using 0.7-kb fragments of *L. jeonii sams* gene as the probe (Fig. 2B). When DNA was digested with *Dpn*I and *Sau*3AI, a 520-bp fragment was detected, but higher molecular-mass bands in *Dpn*II digests, indicating dam-site methylation in *L. jeonii sams*. *Dpn*I recognizes the GATC sequence but cleaves only if the internal A is methylated.

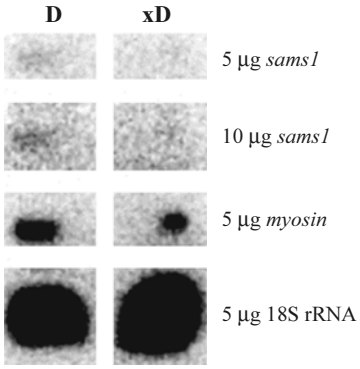

Fig. 1. Run-on assay. Nuclei of D or xD amoebae were used in the *in vitro* transcription. The ^{32}P-labeled RNA was hybridized to Hybond nylon membrane with immobilized spots containing 5 and 10 μg of *sams1*, *myosin*, and 18S rRNA of amoebae, respectively.

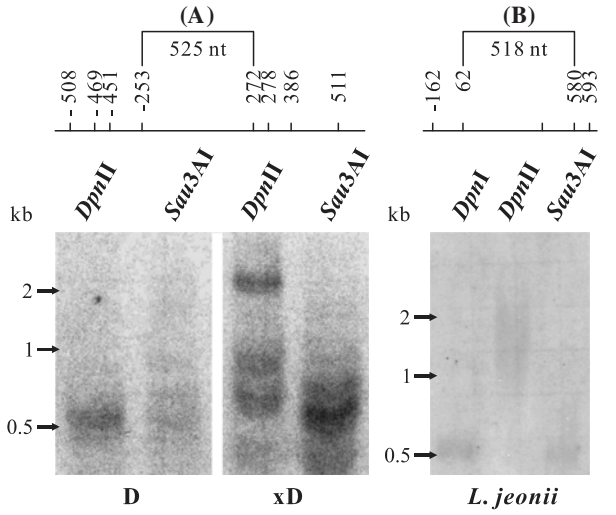

Fig. 2. Southern-blot analysis for DNA adenine methylation. The DNA of D and xD amoebae (A) or *L. jeonii* (B) was analyzed after digestion with *Dpn*I, *Dpn*II, and *Sau*3AI. The restriction map of the 5'-end promoter region of amoeba *sams1* and *L. jeonii sams* shows recognition sites of the restriction enzymes and the sizes of fragments detected in blots.

In vitro transcription using methylated and unmethylated amoeba *sams1* genes

To determine if dam-site methylation of *sams1* in xD amoebae caused inactivation of the gene, the transcripts of dam-site methylated and unmethylated *sams1* genes by *in vitro* transcription assays using D and xD amoeba nuclear extracts were compared (Fig. 3). In the assay using D-amoeba nuclear extracts, there was no noticeable difference in transcription of *sams1* between methylated and unmethylated DNAs. Both methylated and unmethylated *sams1* showed a major band of 110 bp corresponding to the size of the fragment transcribed from the transcription start point (nt -56) of *sams1* reported previously by Jeon and Jeon (2003). The DNA piece without the promoter region of *sams1* showed

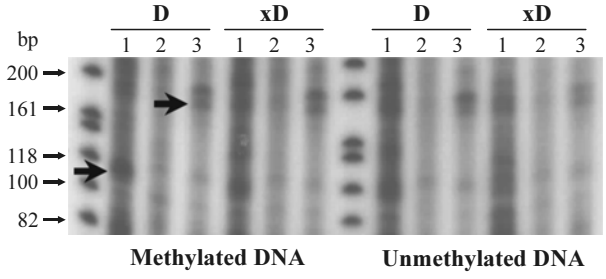

Fig. 3. *In vitro* transcription assay using amoeba nuclear extracts. Methylated and unmethylated amoeba *sams1* genes (lane 1), located from nt -530 to 1212, were used as DNA templates. The amoeba *sams1* gene (nt 106~1212) was used as a negative control (lane 2) and actin promoter 15 of *Dictyostelium* was used as a positive control (lane 3). Arrows indicate transcripts of *sams1* and *actin*, respectively. As a size marker, ϕx174 DNA/Hif1 (Promega) was end-labeled with [γ-^{32}P] ATP using the T4 polynucleotide kinase.

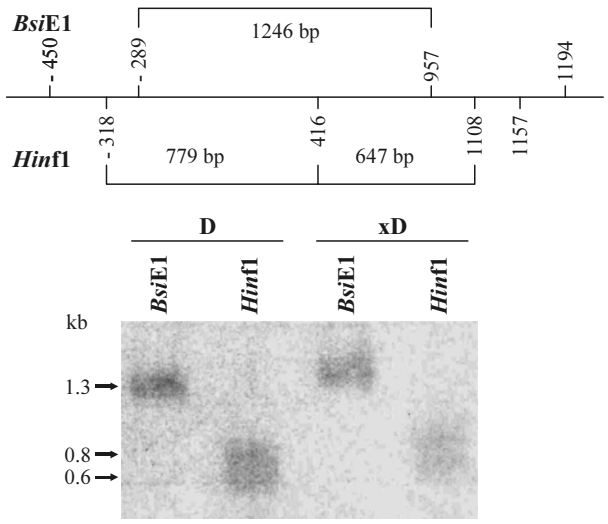

Fig. 4. Southern-blot analysis for the CpG methylation in *sams1*. D and xD amoeba genomic DNAs were analyzed after digestion with *Bsi*E1 or *Hin*fI. The full-length *sams1* gene was used as a probe. The restriction map shows recognition sites of *Bsi*E1 and *Hin*fI.

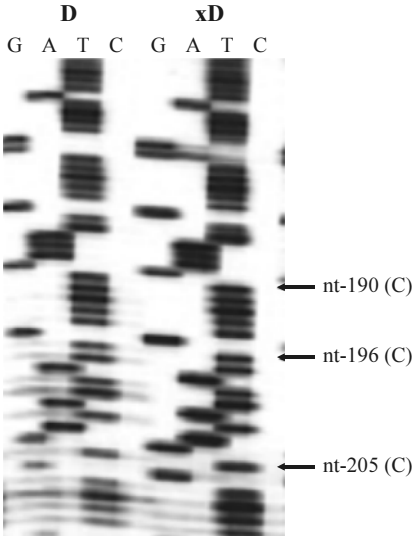

Fig. 5. Bisulfite genomic sequencing for the promoter region of *sams1*. Bisulfite-treated D and xD amoeba *sams1* genes were amplified and sequenced (see 'Materials and Methods'). Arrows indicate cytosine residues of CpG sites converted to thymine in both D and xD amoebae.

no band and actin DNA used as a positive control showed a band of 160 bp.

In *in vitro* transcription using xD-amoeba nuclear extracts, both methylated and unmethylated *sams1* genes also showed a band of same size detected in the assay using D-amoeba nuclear extracts. This result suggests that the region for negative regulation of *sams1* might be present outside the gene used in these experiments since the *sams1* gene was completely suppressed in xD amoebae.

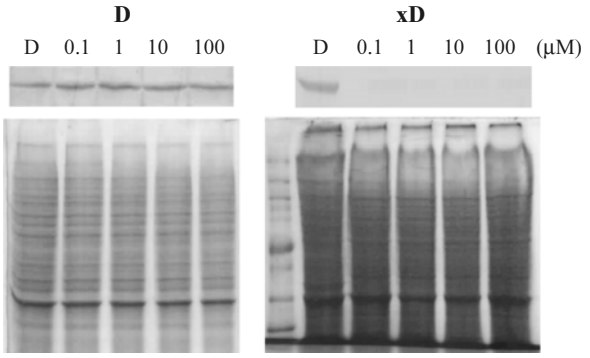

Fig. 6. Western-blot analysis for the expression of *sams1* after treating amoebae with 5-azacytidine at various concentrations for 6 days. The expression level of *sams1* was determined by immunoblotting with a pAb against amoeba-SAMS1.

Analysis for CpG methylation in *sams1*

DNA-digestion analysis using CpG methylation-sensitive restriction enzymes *Bsi*EI and *Hin*f1 was performed to check if silencing of the *sams1* gene in xD amoebae was accompanied by changes in CpG methylation of the gene. Cleavage of DNA with *Bsi*EI or *Hin*f1 restriction enzyme are blocked by CpG methylation. As seen in Fig. 4, *BsiE*1 treatment of D and xD genomic DNA showed a same-size band of 1.3 kb. There were four recognition sites for *Hin*f1 in *sams1*, and two bands with sizes of approximately 0.8 and 0.7 kb were detected in *Hin*f1 digests of D and xD genomic DNAs. These results indicate that the recognition sites of *Bsi*E1 and *Hin*f1 in *sams1* were not methylated.

To test methylation states of other CpG sites in the 5'-end region, bisulfite genomic sequencing was performed The methylation states of 18 CpG sites out of 21 sites in the 5'-end region of *sams1* were examined and no methylated CpG site in both D and xD amoebae was found. Fig. 5 shows only three cytosine residues of CpG sites converted to thymines.

In order to determine if methylation of CpG sites plays a role in *sams1* silencing in xD amoebae, the methylation status of amoebae was modified by treating them with the demethylating agent, 5-aza-2'-deoxycytidine (5-Aza-CdR). For this purpose, amoebae were grown in various concentrations of 5-azadeoxycytidine for 6 days and assessed the amounts of SAMS1 by Western blotting using an anti-SAMS1 antibody (Fig. 6). Under these conditions, there was no change and no reactivation of *sams1* expression in D and xD amoebae. Taken together, these results indicate that CpG methylation are not involved in the inactivation of the *sams1* gene in xD amoebae.

Regulation of amoeba *sams* genes

In order to see if the expression of amoeba *sams* genes was restored to a normal state after the removal of *L. jeonii* symbionts, RT-PCR analysis using total RNAs extracted from D and xD amoebae after removing *jeonii* by growing amoebae for 8 days at 27°C was performed (Fig. 7). After growing for 8 days at 27°C, D amoebae showed no change in the expression of *sams1* and *sams2* compared with those grown at the normal temperature. In contrast, a slight re-

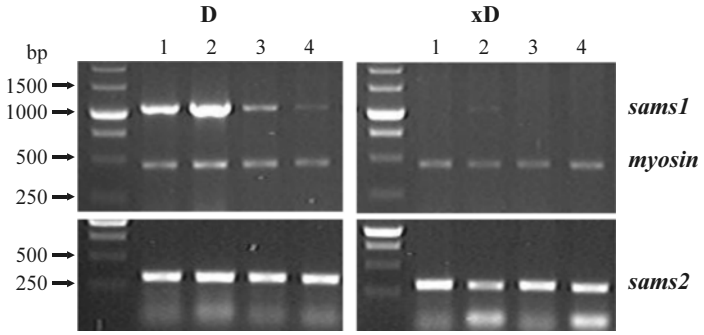

Fig. 7. RT-PCR analysis for *sams* genes. Total RNAs of amoebae were analyzed with *sams1*-, *sams2*-, or *mysosin*-specific primers after growing them for 8 days at 27°C (lane 2) and treating with 1-mM SAM for 3 days (lane 3) and 1-mM Met for 2 days (lane 4), respectively. Amoebae with no treatment were used as controls (lane 1).

expression of *sams1* was detected in RT-PCR in xD amoebae after the removal of *L. jeonii* even though no SAMS1 was detected by Western blotting using an anti-SAMS1 antibody (data not shown). In addition, the *sams2* gene was down-regulated in xD amoebae after the removal of *L. jeonii* (Fig. 7).

In order to study the regulation of amoeba *sams* genes by SAM or Met, the expression levels of *sams* genes were measured after SAM or Met treatment by RT-PCR (Fig. 7). Treatment with SAM or Met had the same effect on the expression of *sams* genes. The expression of *sams1* in D amoebae was down-regulated by SAM or Met treatment, but there was no change in the expression of *sams2*. In xD amoebae, no *sams1* transcript was detected by RT-PCR after treatment with SAM or Met. Also, there was no difference in the expression of *sams2* in xD amoebae following treatment with SAM or Met as compared with control. These data indicated that SAM and Met play a role in the regulation of *sams1* as a negative regulator, but with no effect on that of *sams2*.

Discussion

The examination of methylation status of the *sams1* promoter in this study showed that the *sams1* gene of amoebae was methylated at an internal adenine residue of some GATC sites in symbiont-bearing xD amoebae, but no methylation occurred in symbiont-free D amoebae. This appears to be the first reported case where DNA adenine methylation status of a specific host gene is shown to change during establishment of symbiosis. The modification might be a reason for the inactivation of *sams1* in xD amoebae. The *in vitro* transcription assay using amoeba nuclear extracts and methylated or unmethylated amoeba *sams1* genes indicated that the negative regulatory region for the inactivation of *sams1* was not located on the *sams1*-DNA template (from nt -530 to 1212) used in these experiments. It may be located outside the gene since *sams1* is completely suppressed in xD amoebae (Choi *et al*., 1997; Jeon and Jeon, 2004). The run-on assay using amoeba nuclei showed that the *sams1* gene is inactivated at the transcriptional level in xD ameobae.

DNA adenine methylation plays an important role in regulating the initiation of DNA replication and mismatch repair, phase variation, and gene regulation, host-pathogen interactions (Heithoff *et al.*, 1999; Wion and Casadesus, 2006; Heusipp *et al*., 2007). In this study, the results showing that a host-*sams1* gene is methylated at adenine residues at dam sites during symbiotic interactions between ameobae and *L. jeonii* suggest that the modification might be involved in the inactivation of the *sams1* expression in xD ameobae. This may contribute to the establishment of symbiosis. Recent reports suggest that DNA adenine methylation is involved in the development of symbiosis since the bacterial DNA adenine methylation status changes dramatically during the establishment of symbiosis between *Mesorhizobium loti* and host plant roots (Ichida *et al*., 2007).

DNA adenine methylation is catalyzed by DNA adenine methyltransferase (Dam), encoded by the *dam* gene. Dam methylases are found in several strains of Gram-positive and Gram-negative bacteria (Wion and Casadesus, 2006; Heusipp *et al*., 2007). It has been reported that DNA adenine methylation is also present in *Legionella* (Lema and Brown, 1996) and the methylation in *Legionellae* may play a role in controlling the piliation and virulence of these organisms (Lema and Brown, 1996). Present data show that adenine residues in *L. jeonii sams* are also methylated. In the BLAST search in the NCBI/BLAST website with the amino acid sequence of *Escherichia coli* DNA adenine methyltransferase (gi: 119389532), a highly homologous protein (gi: 52840256) was identified in *Legionella pneumophila* strain, 46% identical to *E. coli* Dam methyltransferase. Taken together with the result of dam-site methylation analysis, these data suggest that *L. jeonii* symbionts are dam-positive strains.

As a result of harboring *L. jeonii* bacteria, xD amoebae become dependent on their symbionts for survival. Nuclei of xD amoebae are no longer compatible with the cytoplasm of D amoebae, the original strain, and established xD amoebae die if their symbionts are removed (Jeon and Ahn, 1978). These observations indicate that irreversible changes have occurred in symbiont-bearing xD ameobae. It is possible that Dam methytransferase of *L. jeonii* is transported to their host amoebae, modulating the regulation of some essential host genes including *sams1* by DNA adenine methylation. It is known that the DNA adenine methylation is a common modification in bacterial genomes. In the amoeba-bacteria

symbiosis, a protein of *L. jeonii* is known to be transported to host amoebae (Pak and Jeon, 1996, 1997). The 29-kDa protein (S29x) is produced by *L. jeonii* and transported to the host-amoeba cytoplasm and the amoeba's nucleus. The function of S29x is not clearly known but the protein is suspected to regulate a gene involved in bacterial infectivity and/or their intracellular survival (Pak and Jeon, 1996, 1997). It is also possible that Dam methylases of host amoebae are activated after infection with *L. jeonii,* subsequently methylating the *sams1* gene. It is desirable to know which organism is the source of Dam methylase in amoeba-*L. jeonii* symbiosis in elucidating the mechanism of DNA adenine methylation.

All Dam methylases use SAM as a methyl donor (Wion and Casadesus, 2006; Heusipp *et al*., 2007). SAM is known not only as a major methyl donor but also as the precursor for biotin, spermidine, and polyamine (Thomas and Surdin-Kerjan, 1997; Mato and Lu, 2007). Additionally, SAM is an intracellular signal to control several essential cellular functions and regulation of *sams* gene itself. It has been reported that the expression of the two *SAM* genes in yeast is regulated differently, *SAM2* being induced by the presence of excess methionine in the growth medium and *SAM1* being repressed under the same conditions (Thomas and Surdin-Kerjan, 1997). In amoebae, it appears that SAM and Met act as a negative regulator for the expression of *sams1*. It is unknown how the *sams1* gene is down-regulated by SAM and Met treatment, but specific transcription factors and/or binding sites might be considered to be important for the down-regulation of the gene. Interestingly, after the removal of X-bacteria from xD amoebae by growing for 8 days at 27°C, the *sams1* gene was slightly reactivated and *sams2* was down-regulated. The results are reversed in the expression of the two *sams* genes when amoebae are infected with X-bacteria. In Western blot analysis, there was no re-expression of *sams1* even after X-bacteria had been removed from xD amoebae. The discrepancy between protein and RNA levels in the expression of *sams1* after the removal of X-bacteria is due probably to different sensitivities of the experimental procedures.

Present study shows a difference in DNA adenine methylation status of the *sams1* gene between D and xD amoebae. In addition, the data in this study indicate that CpG methylation is not responsible for the suppression of *sams1* in xD amoebae. It was previously shown that there was no DNA-sequence difference in the *sams1* gene between D and xD amoebae (Jeon and Jeon, 2003). It appears that the expression of *sams1* in xD amoebae is suppressed by a novel mechanism, probably DNA adenine methylation, as in other eukaryotic gene regulation. Further work is needed to determine which dam sites in the *sams1* gene in xD amoebae are methylated and what the roles of the methylation are in the suppression of *sams1*. The mechanism for the suppression of amoeba *sams1* by *L. jeonii* would be a good example for the alteration of host gene expression brought about by interactions between hosts and infective agents in general.

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