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# Exocyclic GpC DNA methyltransferase from *Celeribacter marinus* IMCC12053§

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# *Celeribacter marinus* IMCC12053의 외향고리 GpC DNA 메틸트랜스퍼라아제§

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DNA methylation is involved in diverse processes in bacteria, including maintenance of genome integrity and regulation of gene expression. CcrM, the DNA methyltransferase conserved in Alphaproteobacterial species, carries out $N^6$-adenine or $N^4$-cytosine methyltransferase activities using S-adenosyl methionine as a co-substrate.

*Celeribacter marinus* IMCC12053 from the Alphaproteobacterial group was isolated from a marine environment. Single molecule real-time sequencing method (SMRT) was used to detect the methylation patterns of *C. marinus* IMCC12053. Gibbs motif sampler program was used to observe the conversion of adenosine of 5'-GANTC-3' to $N^6$-methyladenosine and conversion of $N^4$-cytosine of 5'-GpC-3' to $N^4$-methylcytosine. Exocyclic DNA methyltransferase from the genome of strain IMCC12053 was chosen using phylogenetic analysis and $N^4$-cytosine methyltransferase was cloned. IPTG inducer was used to confirm the methylation activity of DNA methylase, and cloned into a pQE30 vector using *dam-/dcm- E. coli* as the expression host. The genomic DNA and the plasmid carrying methylase-encoding sequences were extracted and cleaved with restriction enzymes that were sensitive to methylation, to confirm the methylation activity. These methylases protected the restriction enzyme site once IPTG-induced methylases methylated the chromosome and plasmid, harboring the DNA methylase. In this study, cloned exocyclic DNA methylases were investigated for potential use as a novel type of GpC methylase for molecular biology and epigenetics.

**Keywords:** adenosine, cytosine, DNA methyltransferase, exocyclic amine group

Bacterial DNA methylation is one of the key steps in the maintenance of genome integrity and regulation of gene expressions (Gonzalez *et al*., 2014). DNA methylates due to formation of $N^6$-methyladenine and 5-methylcytosine by an enzyme DNA methyltransferase (MTase), which plays important roles in gene transcription, bacterial growth, and proliferation (Li *et al*., 2017). Genes involved in DNA methylation and DNA methyltransferases (MTases) have been studied for the last 70 years (Jurkowska and Jeltsch, 2016). Deoxyadenosine methyltransferase (*Dam*) and *CcrM* (cell cycle-regulated MTase) have been widely studied and researched. Dam is important for the expression of pap operon, DNA replication initiation, and DNA repair (Adhikari and Curtis, 2016). *CcrM* is well-conserved among the Alphaproteobacterial lineage (Gonzalez *et al*., 2014), and *CcrM*-mediated DNA-methylation is essential in cell-cycle

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regulation (Mohapatra *et al*., 2014).

*Caulobacter crescentus* NA1000 is a model microorganism for gene expression study of *CcrM* protein (Kozdon *et al.*, 2013), and CcrM homologs as other alphaproteobacterial DNA-(adenine $N^6$)-MTases have gained attention (Maier *et al.*, 2015). CcrM protein carries out cytosine-$N^4$-MTase activity by using S-adenosyl methionine as a co-substrate, and also transforms $N^6$-methyl adenosine (5'-GANTC-3') (Jeltsch *et al.*, 1999). $N^6$- or $N^4$-methyltransferase activities of CcrM-type proteins are grouped as exocyclic amino moiety enzymes compared to 5-methyl cytosine modifications, which directly modify the heterocyclic aromatic ring of the pyrimidine derivative (Loenen and Raleigh, 2014).

*Celeribacter marinus* IMCC12053 and *Novosphingobium pentaromativorans* US6-1 were isolated from a marine environment (Baek *et al.*, 2014), and the strain IMCC12053, which is a known host for marine bacteriophage P12053L, was selected (Kang *et al.*, 2012). *N. pentaromativorans* US6-1 was originally isolated as an oil spill degrading bacterium (Sohn *et al.*, 2004) and the genome was elucidated form mining aromatic-hydrocarbon-degrading genes (Luo *et al.*, 2012; Choi *et al.*, 2015). Both the strains *C. marinus* IMCC12053 and *N. pentaromativorans* US6-1 were known to have adenosine-specific DNA methylase and $N^4$-cytosine specific DNA methylase, according to our previous study (Yang *et al.*, 2016). The CcrM homologs, IMCC12053_18853 (GenBank Acc. ALI55832) from strain IMCC12053 and AIT78768 (GenBank Acc. AIT78768) from US6-1, showed GpC and CpG motif-specific exocyclic amine methylating activities, respectively (Yang *et al.*, 2016).

In the present study, we report the cloning and characterization of exocyclic DNA MTase from *C. marinus* IMCC12053, using *N. pentaromativorans* US6-1 genes as a control.

## Materials and Methods

### Methylation pattern analysis

Single molecule real time (SMRT) sequencing data produced by PacBioRS II were used for finding patterns from genomes of *C. marinus* IMCC12053 (Yang *et al.*, 2017) (Table 1) and *N. pentaromativorans* US6-1 (data not shown). In this study, SMRT method (Eid *et al.*, 2009) was exploited to find patterns of DNA methylation from the strains. Methylated patterns were analyzed by Gibbs motif sampler program (Lawrence *et al.*, 1993), and we could obtain methylation patterns covering GpC (Yang *et al.*, 2017) or CpG regions (Unpublished data by Yang, Jhung Ahn). IMCC12053_18853 (GenBank Acc. ALI55832) from *C. marinus* IMCC12053 and other related sequences of *CcrM* homologs were used for phylogenetic analyses using amino acids or nucleotide alignments. Amino acid sequences were aligned using hmm-aligner from hmmer 3.0 program (Eddy, 2011) and RAxML (ver. 7.3.4) (Stamatakis, 2006) were used for constructing the generation tree. Nucleotide alignments and subsequent analysis using MEGA7.0 program (Kumar *et al.*, 2016) and MrBayes 3.2 (Ronquist *et al.*, 2012) were used for constructing the Bayesian inference tree.

### Strains and media

*C. marinus* IMCC12053 and *N. pentaromativorans* US6-1 cultures were used for extracting genomic DNA. Cloning vectors used for open reading frames from the genomic DNA were pGEMT-Easy and pGEMT Vector Systems (Promega). Expression of the DNA methylase was confirmed by sub-cloning into pQE30 (Qiagen). Chemically competent *E. coli* strains DH5α (Enzynomics) and HIT™-GM2163 (*dam*$^-$/*dcm*$^-$*E. coli* K12 strain, HIT™-GM2163 Value $10^8$, RBC) were used for the study. HiYield Plus™ Plasmid Mini Kit (RBC) was used for plasmid DNA purifications. Luria-Bertani (LB) medium (Difco) was used with or without 100 μg/ml ampicillin. LB agar plates were also used with 100 μg/ml ampicillin plus 15 g/L Bacto Agar (Difco).

### Genomic DNA extraction and polymerase chain reaction (PCR)

Genomic DNAs were purified using PureHelix Genomic DNA Prep Kit (Column type) (NanoHelix) and genomic DNAs were the template for PCR. Sequence-specific forward and reverse oligonucleotides for DNA methylases were as follows: 5'-TGACGACGAAAACACGTGAGGC-3'/5'-AGTTCATC TCCGCGCGGATTTG-3' for *C. marinus* strain IMCC12053 and 5'-TGGGGCAGGTACTCGTCAAGG-3'/5'-ACGGCTC GGTGGCAAGCAGG-3' for *N. pentaromativorans* strain US6-1.

SimpliAmp Thermal cycler (Life Technologies) was used

**Table 1. Sequence motif elements flanking m4C ($N^4$-methylcytosine) and m6A ($N^6$-methyladenosine) modifications in the IMCC12053 genome**
Gibbs program (v3.1) was used for collecting DNA sequences (10 base pairs long window size) and motif elements occurring greater than 50% of the time was used for visualization of the patterns. Methylated bases are shown in black letters.

| Motif element | Left end | Right end | No. motifs | Avg. score (-10 LogP) | Standard deviation |
|---|---|---|---|---|---|
|  | -9 | 0 | **43** | 25.7 | 4.81 |
|  | -8 | 1 | **44** | 24.7 | 4.30 |
|  | -7 | 2 | **70** | 25.1 | 5.81 |
|  | -6 | 3 | **10** | 23.4 | 2.76 |
|  | -5 | 4 | **439** | 25.4 | 5.34 |
|  | -3 | 6 | **172** | 25.7 | 5.38 |
|  | -2 | 7 | **19** | 25.3 | 5.69 |
|  | -1 | 8 | **9** | 28.1 | 9.55 |
|  | 0 | 9 | **19** | 27.8 | 6.24 |
|  | -9 | 0 | **65** | 51.9 | 24.2 |
|  | -8 | 1 | **35** | 51.9 | 22.7 |
|  | -4 | 5 | **1537** | 59.8 | 17.7 |
|  | -3 | 6 | **1110** | 59.3 | 16.9 |
|  | -2 | 7 | **11** | 26.2 | 5.81 |
|  | -1 | 8 | **9** | 21.8 | 2.33 |
|  | 0 | 9 | **8** | 25.5 | 4.17 |

for PCR. Initial denaturation was done for 30 sec at 95°C, amplification comprising the three-step temperature cycles of 30 sec at 95°C, 30 sec at 58–60°C, and 60 sec at 72°C was iterated 25 times. Final primer extension was done by incubating tubes for 60 sec at 72°C. Amplified PCR fragments were confirmed on 1% agarose gel by SYBR Safe DNA Gel Stain (Invitrogen). DNA fragments were cloned into pGEMT-Easy or pGEMT vectors. Cloned sequences were finally confirmed using Big Dye Sanger sequencing by Macrogen Inc.

## Restriction enzyme digestion for sub-cloning open reading frames

DNA methylase ORF (GenBank Acc. ALI55832) from *Celeribacter marinus* IMCC12053 was digested with *Kpn*I,

*Pst*I, and ORF (GenBank Acc. ALI55832) for *Novosphingobium pentaromativorans* US6-1 (GenBank Acc. AIT78768) was treated with *Sph*I and *Sal*I. Cloning vector pQE30 was used for the sub-cloning of the DNA fragments. The vector was treated with *Kpn*I/*Pst*I or *Sph*I/*Sal*I, followed by treatment with shrimp alkaline phosphatase (SAP) for 30 min at 37°C. SAP reaction was stopped by heat-inactivation at 65°C for 5 min. T4 DNA ligase (Promega cat. M180A) and accompanying standard sticky end ligation was done for ligating vectors and DNA inserts. Ligation reaction mixtures were transformed into competent DH5α (Enzynomics).

### DNA methylase assay using methylation-sensitive restriction digestion

Once ALI55832 (IMCC12053_18853 from *C. marinus* IMCC12053) and AIT78768 (from *N. pentaromativorans* US6-1) were sub-cloned into pQE30 and *E. coli* DH5α, each plasmid was transformed into *E. coli* HIT$^{TM}$-GM2163 that had *dam*$^-$/*dcm*$^-$ phenotypes. DNA methylase carrying the expression vector would methylate pQE30/methylases and chromosomal DNAs, once the cultures were treated with 100 μM IPTG for 3 h at 37°C in a shaking incubator (250 rpm).

Methylated and pristine genomic DNAs were purified using PureHelix Genomic DNA Prep Kit (Column type) (NanoHelix) using 4 ml of *E. coli* cultures. Plasmids were also prepared using a HiYield plus plasmid mini Kit (RBC), using 3 ml of the cultures.

Enzymes for methylation sensitive restriction digestion included *Mbo*I, *Msp*I, and *Mlu*I (NEB). Methylated and non-methylated genomic DNAs and pQE30 plasmids carrying DNA methylases were treated at 37°C for 2 h; thereafter, these were analyzed on a 1% or 1.2% agarose TAE gel electrophoresis system and the gels were photographed after staining with SYBR safe DNA gel stain.

## Results and Discussion

### Methylation pattern recognition using PacBio RS II data

According to SMRT (single-molecule, real-time) analysis and Gibbs motif sampler program analysis (Yang *et al.*, 2017), *C. marinus* IMCC12053 showed N$^4$-cytosine methylase activity (GpC methylase) in addition to N$^6$-adenosine methylase activity (5'-GANTC-3') (Table 1).

CDD (Conserved Domain Database) search program of NCBI (Marchler-Bauer *et al.*, 2015) indicated that IMCC12053_18853 (GenBank Acc. ALI55832) could be annotated as *CcrM* (CCNA_00382) from *Caulobacter vibrioides* NA1000 and these proteins could be observed for other genomes (Table 2). Using *N. pentaromativorans* US6-1 genome analysis as a control, we could also annotate AIT78768 as another *CcrM* (Table 2) with N4-cytosine methylase activity (CpG methylase, Supplementary data Table S1).

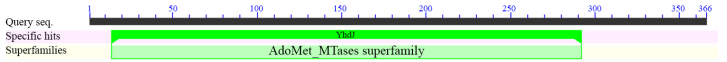
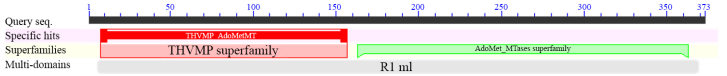
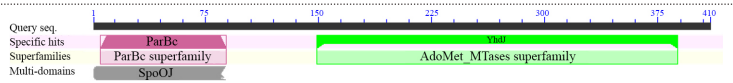
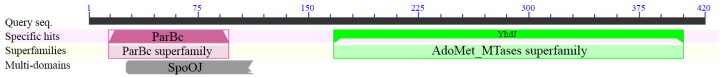
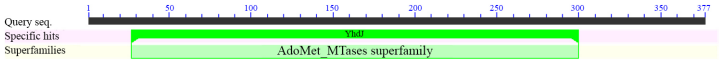
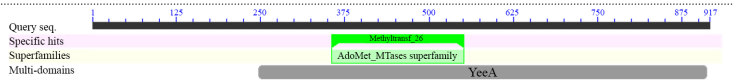
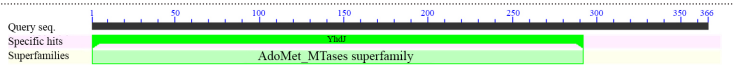
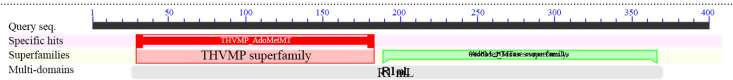
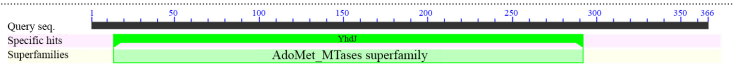
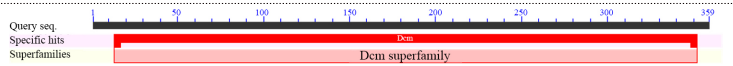
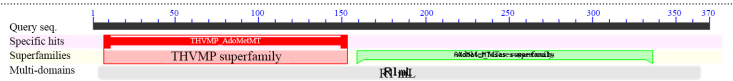
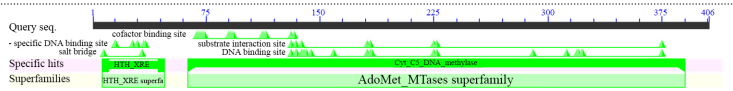
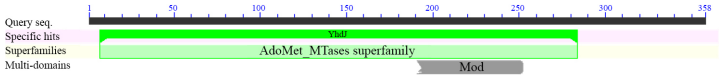
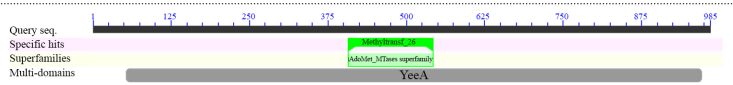
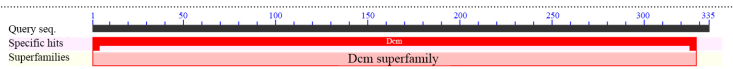
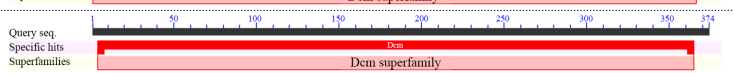
### Phylogenetic analysis of DNA methylases from *Celeribacter marinus* IMCC12053 and related bacteria

We selected DNA methylases from *C. marinus* IMCC12053 and other genomes for constructing phylogenetic trees. To begin with, *C. marinus* IMCC12053 DNA methylases were four in number (Table 1) and these sequences were used as seed sequences for hmm search from hmmer 3.0 program. Using archaeal and bacterial sequences as outgroup sequences, CcrM related amino acids were aligned for a phylogenetic tree using RAxML (ver. 7.3.4) (Stamatakis, 2006). Alphaproteobacterial CcrM sequences were grouped with high boot strap value (94%) with CcrM from *Caulobacter crescentus* NA1000; others with EC 2.1.1.113 were methylases with N$^4$-cytosine MTase activities. RAxML analysis was done with 29 amino acid sequences with 431 distinct alignment patterns and substitution matrix was LG. Gaps and undetermined characters were excluded from the alignmnet, 161 amino acids were used for the RAxML with 100 rapid bootstrap values (Fig. 1).

As shown in Fig. 1, 9 *CcrM* sequences from the upper-most part of the tree includes *C. marinus* IMCC12053 and *N. pentaromativorans* US6-1. These sequences formed a distinct clade with *C. crescentus* NA1000 with a boot strap value of 94%.

Using nucleotide sequence of IMCC12053_18853 (GenBank Acc. ALI55832 from CP012023.1) as a seed sequence for blastn and MEGA7.0 program for alignments, optimal condition determination, and nexus format file export for MrBayes. The 29 sequences collected, were used for Bayesian inference tree construction using MrBayes 3.2. IMCC12053_18853 (from *Celeribacter marinus* IMCC12053) formed a distinct sister-group

**Table 2. Comparison of DNA methyl transferases among some strains from Alphaproteobacteria subdivision**

| Organism | Locus tag | Description | CDD result | Comment |
|---|---|---|---|---|
| *Celeribacter marinus* IMCC12053 | IMCC12053_1885 | DNA modification methylase |  | *CcrM* |
| | IMCC12053_605 | Predicted $N^6$-adenine-specific DNA methylase |  | |
| | IMCC12053_2040 | DNA modification methylase |  | |
| | IMCC12053_3116 | DNA modification methylase |  | |
| *Novosphingobium pentaromativorans* US6-1 | AIT78768 | DNA modification methylase |  | restriction endonuclease subunit M |
| | AIT79233 | Type II restriction enzyme, methylase subunit YeeA |  | Annotated as “lactate dehydrogenase” |
| *Celeribacter baekdonensis* B30 | B30_03115 | Adenine-specific methyltransferase |  | *CcrM* |
| | B30_06546 | Predicted $N^6$-adenine-specific DNA methylase |  | |
| *Celeribacter indicus* P73 | P73_0385 | Adenine-specific methyltransferase |  | *CcrM* |
| | P73_4804 | DNA-cytosine methyltransferase |  | |
| | P73_2233 | Predicted $N^6$-adenine-specific DNA methylase |  | |
| | P73_4799 | C-5 cytosine-specific DNA methylase |  | |
| *Caulobacter vibrioides* NA1000 | CCNA_00382 | Adenine-specific methyltransferase |  | *CcrM* |
| | CCNA_00869 | Type II restriction enzyme, methylase subunits |  | adenine methyltransferase with an associated restriction enzyme domain |
| | CCNA_01085 | DNA-cytosine methyltransferase |  | |
| | CCNA_03741 | DNA-cytosine methyltransferase |  | |

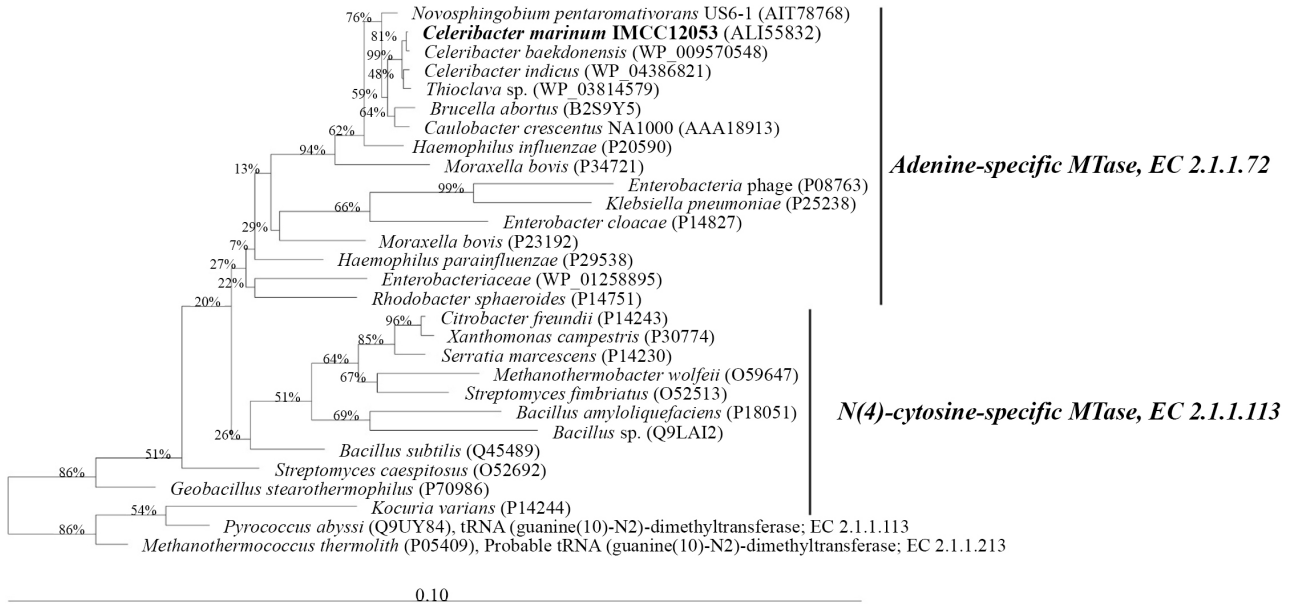



**Fig. 1. Phylogeny of CcrM methyltransferases from Alphaproteobacteria with other groups of bacteria and archaea.** Nine uppermost enzyme sequences represents Alphaproteobacterial CcrM sequences and they grouped with high boot strap value (94%) with CcrM from Caulobacter crescentus NA1000; others with EC number 2.1.1.113 are methylases with $N^4$-cytosine methyltransferase activities and they are listed in REBASE database. Tree was drawn with RAxML (version 7.3.4) with 29 amino acid sequences with 431 amino acid alignment with 100 rapid bootstrap for ML search. Gaps and undetermined characters were 62.69% and 161 amino acids were used for each sequence. Substitution matrix was LG using fixed base frequencies was exploited for the calculation of to an accuracy of 0.1000000000 Log likelihood units.

with *C. crescentus* NA1000, *N. pentaromativorans* US6-1, *Brucella abortus* BAB8416, and *Celeribacter baekdonensis* B30 with 68% probability value (Supplementary data Fig. S1). Phylogenetic analyses with amino acid and nucleotide alignments verified that DNA MTase gene IMCC12053_18853 may play a role as a canonical *CcrM* gene, as seen in *C. crescentus* NA1000 (Adhikari and Curtis, 2016).

## Cloning and expression of IMCC12053_1885

For MTase activity confirmation, we used pristine methylation-free chromosomal or plasmid DNA that was prepared using *E. coli* GSM2163 as a host, but IPTG-induction (100 μM IPTG at 37°C for 3 h) was expected to induce MTase protein expression as well as chromosomal and plasmid DNAs. MTases from strains IMCC12053 and US6-1 were digested using *Msp* I (5'-CCGG-3'; CpG not sensitive) and *Mbo* I (5'-GATC-3'; dam blocked and CpG impaired by overlapping) and MTase expression of US6-1 blocked *Mbo* I site by CpG methylase activities (Supplementary data Fig. S2). From the digestion pattern of GpC or CpG MTases on chromosomal sequences, IMCC12053_1885 (GenBank Acc. ALI55832) was a MTase different from MTase of US6-1. This result corresponded to PacBio RS II data of previous study (Yang *et al.*, 2016).

IPTG-induced and Uninduced plasmid DNA encoding IMCC12053_1885 were digested with CpG blocked restriction enzymes (Supplementary data Table S1) including *Mlu* I (5'-ACGCGT-3') and *Pvu* I (5'-CGATCG-3') according to a previous method (Eberhard *et al.*, 2001). Linearized plasmids were treated with *Pst* I (5'-CTGCAG-3') showed the same pattern for methylated (I) and non-methylated (U) (Fig. 2A). *Mlu* I/*Pst* I digestion showed a slight increase in larger fragment size (that is from a single cut not by *Mlu* I but by *Pst* I) in the IPTG-induced plasmid preparation. *Mlu* I/*Pvu* I digestion produced more evident single fragment (indicated by arrows in Fig. 2A) in the induced plasmid lane. NebCutter 2.0 (Vincze *et al.*, 2003) was used for generating *in silico* digested gel image files (assuming 1.2% TAE agarose) using the pQE30/IMCC12053_1885 sequence (refer to supplementary text) (Fig. 2B). NebCutter 2.0 was used only to calculate CpG methylation and we know that IMCC12053_1885 is not a CpG MTase (Supplementary data Fig. S2), but CpG-blocked *Pvu* I and *Pst* I (not sensitive to any methylation) successfully linearized pQE30/ IMCC12053_

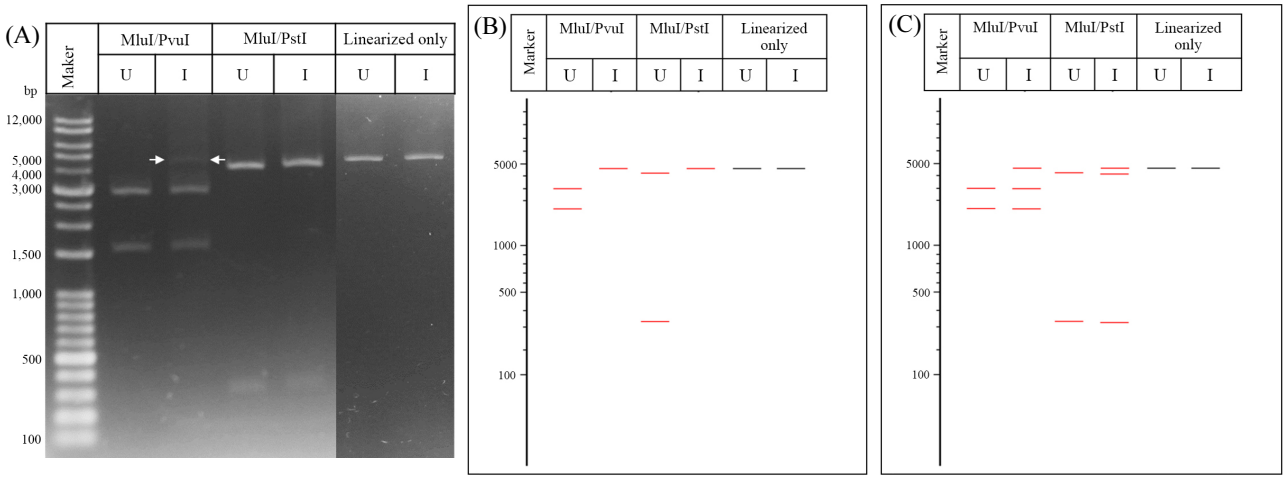



**Fig. 2. Methylation sensitive digestion of plasmid DNA preparations of dam-/dcm- competent *E. coli* GM2163 transformed with IMCC12053_1885 (A).** For linearization, two enzymes were used for digestion. Hemi-methylated or partially methylated bands can be identified in *Mlu* I/*Pvu* I and GpC methylation ( † ) occurred in *Mlu* I (ACG†CGT). *Mlu* I (A*CG*CGT) and *Pvu* I (*CGAT*CG) are both blocked by CpG methylation (*). *Pst* I was not sensitive to CpG/dam/dcm methylation and was used for linearization use only. SYBR stained 1.2% agarose gel with 1 kb Marker from DM3200 (SMOBIO). *U, Uninduced; I, Induced. NEBcutter 2.0 was used for expected DNA framgments from a circular pQE30 vector carrying IMCC12053_1885 sequence (see Supplementary data for the sequence) (B). Actual interpretation of the electrophoresis patterns considering GpC MTase impairment of *Mlu* I (C).

1885. Moreover CpG-blocked Mlu I (5'-ACGCGT-3') also had a GpC dinucleotide recognition to be impaired by IMCC12053_1885 MTase when 100 μM IPTG was added to the culture for expression of MTase activity. In real electrophoresis gels, partially methylated or hemi-methylated fragments from plasmids before and after induction may be observed as seen in Fig. 2A that corresponds to Fig. 2C.

In this study, we cloned and investigated an MTase from *C. marinus* IMCC12053 and it was different from CpG or GpC MTases. Previously reported CpG MTases were from *Spiroplasma* sp. strain MQ1 (*M. SssI*) (Renbaum *et al.*, 1990), and GpC MTases were from *Chlorella* virus NYs-1 (*M. CviPI*) that recognized dinucleotide GpC and methylated 5-methyl cytosines in DNA (Xu *et al.*, 1998). Unlike *M. SssI* and *Chlorella* virus NYs-1 MTases with CpG and GpC activities, GpC MTase from *C. marinus* IMCC12053 is a $N^6$-methyl adenosine or $N^4$-methyl cytosine forming enzyme (Yang *et al.*, 2016). Using phylogenetic analysis, we screened and cloned the gene for exocyclic MTase, IMCC12053_1885 (GenBank Acc. ALI55832) with exocyclic GpC MTases. DNA MTases could be used to protect or impair restriction sites in DNA (Roberts *et al.*, 2015), by changing chemical and physical properties of the DNA (Pérez *et al.*, 2012). Other CpG/GpC MTase studies include methylated-DNA-specific probing and labeling DNA with isotopes (Herring *et al.*, 2009; Harrison and Parle-McDermott, 2011). Most importantly, CpG and GpC MTases are important in gene expression and epigenetic studies (Harrison and Parle-McDermott, 2011; Jang *et al.*, 2017). Recently, GpC MTase, not CpG MTase, was used as a potential regulator of gene expression in mitochondria (van der Wijst *et al.*, 2017), suggesting that alphaproteobacterial DNA MTases may be of importance in the evolution and epigenetics in both bacterial and eukaryotic kingdoms.

## 적 요

DNA 메틸화는 유전체의 무결성의 유지 및 유전자 발현 조절과 같은 박테리아의 다양한 과정에 관여한다. Alphaproteobacteria 종에서 보존된 DNA 메틸 전이 효소인 *CcrM*은 S-아데노실 메티오닌을 공동 기질로 사용하여 $N^6$-아데닌 또는 $N^4$-시토신의 메틸 전이 효소 활성을 갖는다. *Celeribacter marinus* IMCC 12053는 해양 환경에서 분리된 알파프로테오박테리아로서 GpC 시토신의 외향고리 아민의 메틸기를 대체하여 $N^4$-메틸 시토신을 생산한다. 단일 분자 실시간 서열 분석법(SMRT)을 사용하여, *C. marinus* IMCC12053의 메틸화 패턴을 Gibbs Motif Sampler 프로그램을 사용하여 확인하였다. 5'-GANTC-3'의 $N^6$-메틸 아데노신과 5'-GpC-3'의 $N^4$-메틸 시토신을 확인

하였다. 발현된 DNA 메틸전이 효소는 계통 발생 분석법을 사용하여 선택하여 pQE30 벡터에 클로닝 후 *dam*$^{-}$/*dcm*$^{-}$ 대장균을 사용하여 클로닝된 DNA 메틸라아제의 메틸화 활성을 확인하였다. 메틸화 효소를 코딩하는 게놈 DNA 및 플라스미드를 추출하고 메틸화에 민감한 제한 효소로 절단하여 메틸화 활성을 확인하였다. 염색체와 메틸라아제를 코드하는 플라스미드를 메틸화시켰을 때에 제한 효소 사이트가 보호되는 것으로 관찰되었다. 본 연구에서는 분자 생물학 및 후성유전학을 위한 새로운 유형의 GpC 메틸화 효소의 잠재적 활용을 위한 외향고리 DNA 메틸라제의 특성을 확인하였다.

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