ISSN (Online) 2287-3406
Journal of Life Science **2024** Vol. 34. No. 2. 86~93 DOI : https://doi.org/10.5352/JLS.2024.34.2.86

Role of CopA to Regulate *repABC* Gene Expression on the Transcriptional Level

Sam Woong Kim[1], Sang Wan Gal[2], Won-Jae Chi[3], Woo Young Bang[4], Tae Wan Kim[5], In Gyu Baek[6] and Kyu Ho Bang[2]*

[1]*Agri-Food Bio Convergence Institute, Gyeongsang National University, Jinju 52725, Korea*
[2]*Department of Pharmaceutical Engineering, Gyeongsang National University, Jinju 52725, Korea*
[3]*Biodiversity Research and Cooperation Division, National Institute of Biological Resources (NIBR), Environmental Research Complex, Incheon 22689, Korea*
[4]*Biodiversity Research and Cooperation Division, National Institute of Biological Resources (NIBR), Environmental Research Complex, Incheon 22689, Korea*
[5]*Leaders in INdustry University Cooperation 3.0, Gyeongsang National University, Jinju 52828, Korea*
[6]*Laboratory of Aquatic Animal Diseases, Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 52828, Korea.*

Received January 12, 2024 /Revised January 26, 2024 /Accepted February 1, 2024

Since replication of plasmids must be strictly controlled, plasmids that generally perform rolling circle replication generally maintain a constant copy number by strictly controlling the replication initiator Rep at the transcriptional and translational levels. Plasmid pJB01 contains three *orf*s (*copA*, *repB*, *repC or repABC*) consisting of a single operon. From analysis of amino acid sequence, pJB01 CopA was homologous to the Cops, as a copy number control protein, of other plasmids. When compared with a CopG of pMV158, CopA seems to form the RHH (ribbon-helix-helix) known as a motif of generalized repressor of plasmids. The result of gel mobility shift assay (EMSA) revealed that the purified fusion CopA protein binds to the operator region of the *repABC* operon. To examine the functional role of CopA on transcriptional level, 3 point mutants were constructed in coding frame of *copA* such as CopA R16M, K26R and E50V. The repABC mRNA levels of CopA R16M, K26R and E50V mutants increased 1.84, 1.78 and 2.86 folds more than that of CopA wt, respectively. Furthermore, copy numbers owing to mutations in three *copA* genes also increased 1.86, 1.68 and 2.89 folds more than that of *copA* wt, respectively. These results suggest that CopA is the transcriptional repressor, and lowers the copy number of pJB01 by reducing repABC mRNA and then RepB, as a replication initiator.

Key words : CopA, copy number, pJB01, point mutation, transcriptional repressor

Introduction

Although plasmid copy number is variety according to types of host bacteria and growth condition of a given host, any particular plasmid has a characteristic copy number within a given host under fixed growth conditions [4, 5, 12~14, 18, 19]. This constant maintenance is achieved by plasmid-encoded control elements that regulate the initiation of the replication. The control systems lead to maintain the rate of replication in a steady state at the average of specific plasmid per cell cycle. Fluctuation deviations for the average copy number in individual cells are accomplished by antisense RNA or counter-transcribed RNA (ctRNA), inhibiting proteins, or DNA sequences that inhibit the initiator or initiation of replication in a dose-dependent way. This inhibition limits the level of active Rep protein, the availability of the primer for leading strand, or the number of active origins [13].

Negatively controlling mechanisms of replication via studies in various systems have been classified to several types of repressors: (1) control by DNA sites to bind initiator protein, Rep protein (F, P1, RK2 and R6K); (2) by antisense RNA (ColE1, R1 and pT181); (3) by both a transcriptional repressor and antisense RNA (pMV158 and pIP501) [3, 13,

***Corresponding author**
Tel : +82-55-772-3396, Fax : +82-55-772-3399
E-mail : khbang0095@gnu.ac.kr

15, 22, 24, 25, 29, 30]. In general, control model of plasmid replication explains how the average plasmid copy number is maintained in a growing population and how this average is corrected.

In the case of plasmids pMV158 and pJB01 to replicate via rolling circle mode, ctRNAs has been proposed to inhibit translation of the *repB* gene by direct interaction with sequences of translation initiation present in the *repB* mRNA [7~ 9, 11, 19, 20, 26]. In addition, a transcriptional repressor of pMV158, CopG, binds to a single promoter for both *copG* and *repB* genes, and then, represses transcription from the promoter [2, 6, 11, 16, 17]. Plasmid pJB01 is a member of pMV158 family [20], and its CopA expects to play a role as a copy number controller of typical rolling circle replication plasmids. Because a deduced amino acid sequence of pJB01 CopA is homologous with Cops of pMV158 family and assumes to contain a ribbon-helix-helix (RHH) motif on secondary structure of CopA.

In this study, DNA binding ability and functional regions of the CopA protein of pJB01 were examined through gel retardation assay and construction of its mutants. In some detail, CopA plays a role as a transcriptional regulator of *repABC* operon through binding to the operator region of its operon. Three point mutants of CopA such as CopA R16M, K26R and E50V were constructed to examine the functional region of CopA. Among these mutants, it was confirmed that the 50^{th} amino acid, glutamic acid, of CopA played the most important role for transcriptional regulation.

Materials and Methods

Bacterial strains and plasmids

Plasmid pJB01 was isolated by some modifications of the alkaline lysis method from pathogenic *Enterococcus faecium* JC1 [27]. *E. coli* BL21 was used as a host bacterium for overexpression of CopA proteins. *E. faecium* JS2, a strain non-harboring pJB01, was used for determination of plasmid copy number and for RT-PCR. pGEX-2T was used as an overexpression vector. For a host selection harboring target plasmids from *E. faecium* JS2, an *ermC* gene (erythromycin resistant methylase C type gene), which originated from the portion of pE194 [28], from plasmid pGKV21was transferred into pJB01 after amplification by PCR, and named pJB01 *ermC*. Ampicillin (50 μg/ml) and erythromycin (5 μg/ml) were used for selection of cloned recombinants such as pBluescript SK (+)/pJB01, pGEX/copA wt, pGEX/CopA mutants (R16M, K26R, E50V), and pJB01 *ermC*.

Purification of GST::CopA

The *copA* gene was PCR-amplified by using two synthetic oligonucleotides, *copA* 5′-end, and *copA* 3′-end (Table 1), and pJB01 as a template. The PCR products were eluted with a gene clean kit (Bio101, USA), inserted into a pGEM-T Easy Vector (Promega, USA), and then reinserted into the expression plasmid vector pGEX digested to *Bam*HI and *Hin*dIII, designed as a pGEX::*copA*. Expression of the pGEX::*copA* in *E. coli* was carried out according to the Amersham pharmacia biotech's catalogue. Ten milliliters of an overnight cultural broth for *E. coli* BL21 carrying pGEX::*copA* were inoculated into 1 L of LB medium containing 50 μg/ml of ampicillin. The inoculated broth was incubated with vigorous shaking at 30℃ until 0.5-1.0 at OD_{600} in a wavelength. IPTG was then added to 1 mM of final concentration and incubated for an additional 4 hr at 30℃. The cultural cells were harvested by centrifugation, and suspended with 100 ml of PBS buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8

Table 1. oligonucleotides used in this study

names	Nucleotide sequences (5'-3')
copA 5′-end	GGATCCATG GCTAGAAAAAATCAG
copA 3′-end	AAGCTTGTACTCATCTTTG CCCTTTC
ORFA1	GGATCCATGGCTGAAAAATCAG
antisense RNA pro 3′-end	AAGCTTTGGATACAGTAAAAACGTG
R16N overlapping 5′-end	GTAACTGTAANGTTTCCTAA
R16N inverse	TTAGGAAACNTTACAGTTAC
K26N overlapping 5′-end	CAAGAGTACANGAAAATACTA
K26N inverse	AGTATTTTCNTGTACTCTTG
E50N overlapping 5′-end	GTTGTTGAAGNAT
E50N inverse	TTCTCATA TNCTTCAACAAC
Operator-5′-F	GGGGGGGGAGGAAGCAATTTTG
copA 5′-F	ATGGCTAGAGAAAAATCAGA
copA 3′-R	CATCTTTGCCCTTTCTCATA

mM KH_2PO_4, pH 7.4). The suspended cells were treated twice at -70℃ and ice bath for freezing and thawing, respectively, and then sonicated for bacterial lysis. The bacterial lysates were centrifuged at 12,000× g for 10 min at 4℃ to remove insoluble cell debris. The supernatant containing soluble proteins was recovered for purification.

Fifty milliliters of the prepared supernatant were mixed with 500 µl (approximately 50% slurry) of glutathione agarose (Pharmacia, USA), and incubated for 1 hr at 4℃ with gentle shaking. The agarose beads were washed by three times with 7 ml of PBS buffer, pH 7.4. The fusion protein was eluted from agarose beads by three times with 1 ml of 50 mM Tris-HCl (pH 8.0) containing 10 mM glutathione. Free glutathione was removed by dialysis against the solution of 20 mM Tris (pH 8.0), 1 mM EDTA, 100 mM KCl, 5 mM DTT and 20 mM $MnCl_2$. Protein concentrations were determined by the method of Bradford, using bovine serum albumin as a standard. The purified proteins were stored at -80℃ (Fig. 1).

Construction of CopA point mutants and purification of the mutated proteins

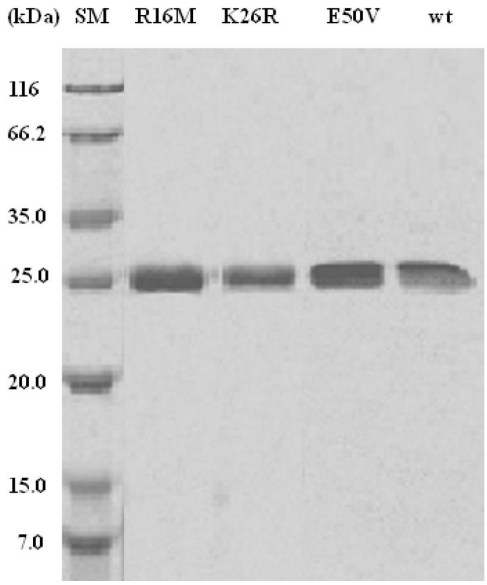

Fig. 1. Purification of wt CopA and its mutants for analysis of functional region. Positional mutants were constructed by CopA R16M, K26R and E50V (R16M; AGG → A**T**G, K26R; AAG → A**G**G, E50V; GAA → G**T**A). Wt CopA and its mutated proteins fused with Glutathione-S-Transferase (GST) were purified by an ordinary method. Electrophoresis was performed by 12% SDS-PAGE and stained with Coomassie brilliant blue R-250. SM, protein molecular weight marker.

Three *copA* point mutants were constructed by overlapping PCR extension [23]. Primers used for all positional mutagenesis were ORFA1 and antisense RNA pro 3′-end (Table 1). R16N overlapping 5′-end and inverse primers were used for R16N, K26N overlapping 5′-end and inverse primers for K26N, E50N overlapping 5′-end and inverse primers for E50N, respectively (Table 1). The constructs were confirmed by DNA sequencing (Sanger's sequencing method). Finally, the constructs were named as CopA R16M, K26R and E50V (R16M: AGG → ATG, K26R: AAG → AGG, E50V: GAA →GTA). Purification of the mutated proteins was performed by the same method as that of pGEX::*copA* wt (Fig. 1).

Gel mobility shift assay of GST-CopA with the DNA stretch containing a putative operator

A DNA segment including a putative operator for the *repABC* operon on plasmid pJB01 was amplified by PCR with operator-5′-F and copA-3′-R. One pmol of each PCR product was incubated with various concentrations of GST-CopA in 20 µl of the reaction buffer [20 mM Tris·HCl, pH 8.0; 1 mM EDTA, pH 8.0; 100 mM KCl; 5 mM DTT; 5 µg/ml calf thymus DNA and 5 µg/ml bovine serum albumin] for 30 min at room temperature. Reactions were stopped by an addition of non-denaturing loading buffer [20% (v/v) glycerol, 0.01% (w/v) bromophenol blue in TBE buffer]. The reaction products were separated on 5% native polyacrylamide gels. The separated bands were stained with ethidium bromide (EtBr; 0.5 µg/ml) in TBE buffer for 30 min, and destained with distilled water for 30 to 120 min.

Analysis of copy number for pJB01 copA mutants

The mutant plasmids were firstly obtained in *E. coli* TG1, and transformed for analyses of copy-number into *E. faecium* JS2. To quantify the copy numbers, *E. faecium* JS2 harboring plasmids were grown for overnight incubation, diluted to 1:25 in prewarmed fresh medium, grown until mid-exponential phase, and then adjusted to 0.5 at A_{600}. Equal aliquots of each culture were withdrawn. Cells were collected by centrifugation and plasmids were prepared by an Accuprep™ plasmid extraction Kit (Bioneer). Extracted plasmids were separated on agarose gels and stained by EtBr (0.5 µg/ml). Covalently closed monomer plasmids were quantified by Gel doc 2000 (Bio-rad) and those of mutant plasmids were compared with that of wt pJB01 *ermC*.

RT-PCR (Reverse transcription-PCR)

For RT-PCR, total RNAs were prepared from exponen-

tially-grown *E. faecium* JS2 cultures harboring pJB01 *copA* mutants by RNeasy Mini kit (Qiagen). Syntheses of cDNAs were done by using superscript II RTase (Gibco-BRL) according to manufacture's directions, and then amplified by general PCR. Primers for RT-PCR were used by *copA* 5′-F and *copA* 3′-R (Table 1).

Results and Discussion

Analysis of CopA structure as a repressor at transcriptional level

Plasmid pJB01 (GenBank accession number AY425961) was isolated from pathogenic bacterium *E. faecium* JC1, and composed of 2,235 bp in length [20]. Factors related with replication including *dso* (double strand origin), *sso* (single strand origin), and three *orfs* were found from its DNA sequence data. The estimated molecular mass of the putative protein encoded by *orfA* was about 6.8 kDa, consisting of 56 amino acids with a calculated pI of 7.93 (Fig. 2). On the DNA sequence level, the CopA showed homology with a Cop which is known as a copy number control protein in other plasmids. When putative secondary structure of CopA was compared with those of Cop homologues by the method of Gomis-Ruth *et al*. [17], a helix-sheet-helix-helix (ribbon-helix-helix) motif known as a DNA binding domain was identified in its structure (Fig. 3). A *repB*, encodes a protein with a molecular mass about 25.5 kDa, consists of 225 amino acids maintaining a theoretical pI value of 6.7. The *repB* is homologous to the members of the Rep proteins, which play critical roles as replication initiators of RCR plasmids, especially of those of pMV158 family. Its function was clearly demonstrated as a replication initiator by our group [20]. The *repC* encodes a protein with a molecular mass about 18.0 kDa, consisting of 153 amino acids, with pI of 5.11, but its potential functional homologue couldn't be addressed due to lack of any significant similarity to previously known proteins via data base search analysis of GenBank [21].

Since consensus sequences of the promoter region were not existed in the intergenic regions of 72 nts (*copA-repB*) and/or 31 nts (*repB-repC*), it was thought that these genes form a single operon. It was demonstrated that these *repABC* consist of single operon by RT-PCR [21]. Thus, If CopA play a role as a repressor at transcriptional level through binding on a putative operator (Fig. 3), expression of RepB, a replication initiator, should be inhibited by CopA, and then copy number of pJB01 decreased by lower level of RepB protein.

CopA to interact with DNA segment including putative operator

To elucidate whether CopA protein on pJB01 is a homologue of other Cop proteins previously known as a repressor,

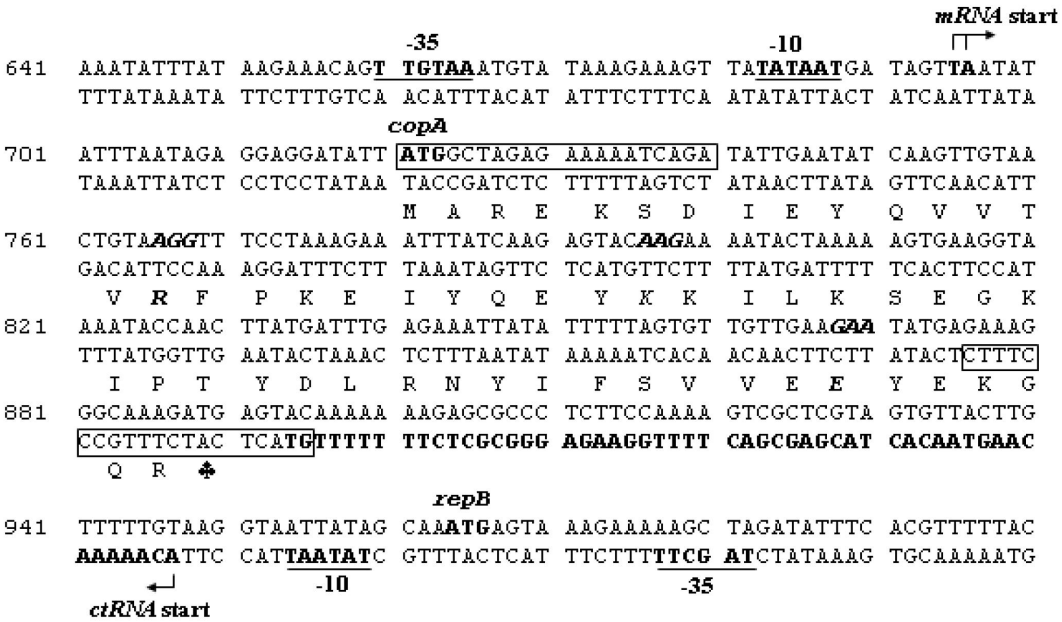

Fig. 2. Putative transcriptional start sites and orientations of *cop*A gene. Arrowheads point in the directions of transcription and the start sites of repABC mRNA and ctRNA, respectively. The –35 and –10 boxes of promoters are shown in underlined boldface letters. The oligonucleotides used for overexpression are boxed.

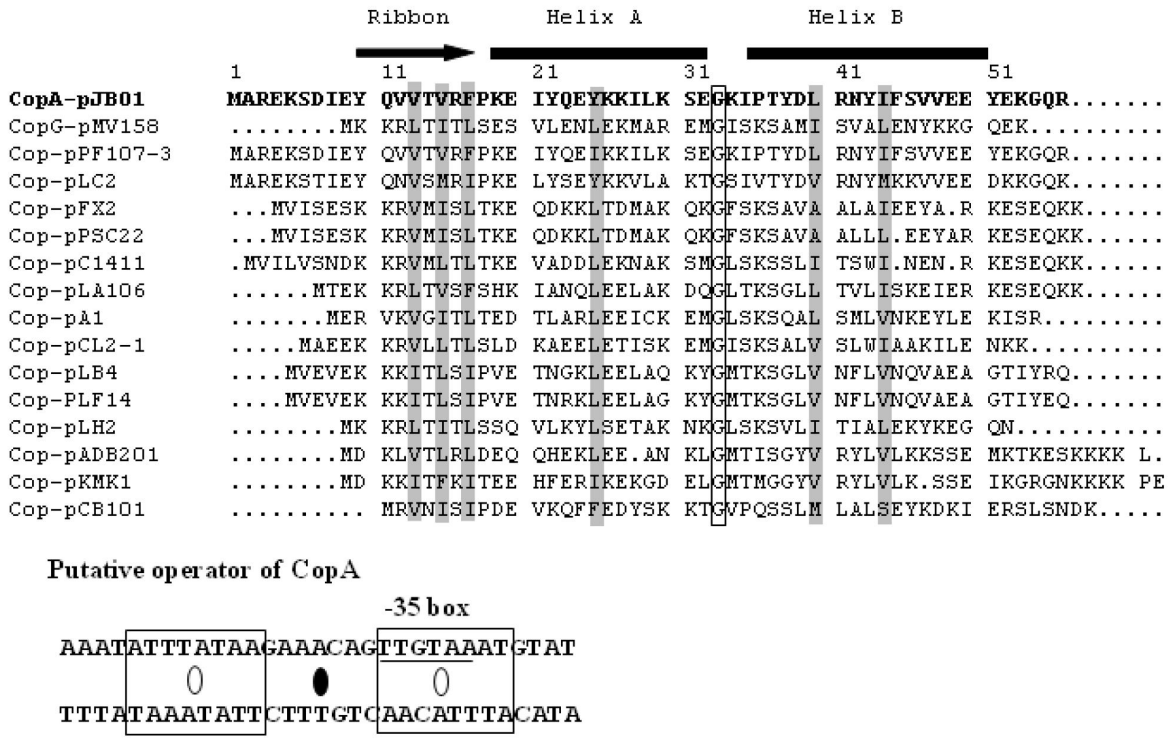

Fig. 3. Comparison of the CopA with other Cops. Amino acid sequences of fifteen members of Cop family are aligned with that of CopA. The boxed glycines are residues of the turn regions connecting helix A and B of a RHH-motif. Conserved (hydrophobic) positions are highlighted by shaded boxes. Putative operator of CopA is composed of a partial symmetrical arrangement including -35 box in promoter of *repABC* operon as indicated on the lower panel.

DNA sequence analysis neighboring *copA* gene, analysis of DNA binding motifs within CopA protein, and EMSA were performed. Gomis-Ruth *et al*. [17] aligned the amino acid sequence of pMV158 CopG with those of 14 Cop components from other plasmids. The results of alignment were suggested that the 15 Cop proteins form a putative ribbon-helix-helix motif (RHH). Furthermore, when pJB01 CopA and pPF107-3 Cop were compared with amino acid sequences, only the 25th Y residue in pJB01 was substituted for I on the same position in pPF107-3 (Fig. 3). Therefore, it could be confirmed that CopA containing RHH motif play a role as a repressor on transcriptional level. In case of pMV158, a transcriptional repressor, CopG, binds to a single promoter for both the *copG* and *repB* genes and represses its transcription [10]. Mutations or deletions in *copG* gene lead to an increase in the plasmid copy number [1], and *copG* exerts a weak incompatibility with the pMV158 replicon when cloned, under its own transcription and translation signals, into compatible vector of a high copy number [11]. To know whether a partial symmetrical DNA sequence including -35 box of *repABC* promoter as shown in Fig. 3 functions as an operator, gel mobility shift assay using pGEX-CopA (Fig. 4) was done. As shown in Fig. 4, a few retarded bands were appeared and amount of retarded DNA was increased by a direct proportion to CopA fusion protein dosage. This result suggests that CopA binds the putative operator and regulates the *repABC* operon on the transcriptional level.

The RHH motif of CopA to play a critical role for interaction with operator

In order to know the functional role of CopA protein in more detail, three point mutants were constructed by arbitrary amino acids of CopA in each domain for identification of critical roles for each motif of ribbon (R16), helix A (K26), and helix B (E50) regions, respectively. In order to examine transcriptional changes by mutations on *copA* gene, RT-PCR was performed by using total RNA isolated from *E. faecium* JS2 harboring pJB01 CopA mutants. When three *copA* mutants were compared with CopA wt on the mRNA level, the *repABC* mRNA expressions by CopA R16M, K26R, and E50V mutants, as shown in Fig. 5, were increased to 1.84, 1.78, and 2.86 folds more than that of CopA wt, respectively. Mutations on RHH motif of CopA led to increase repABC transcript of pJB01. It was deduced that mutations of CopA

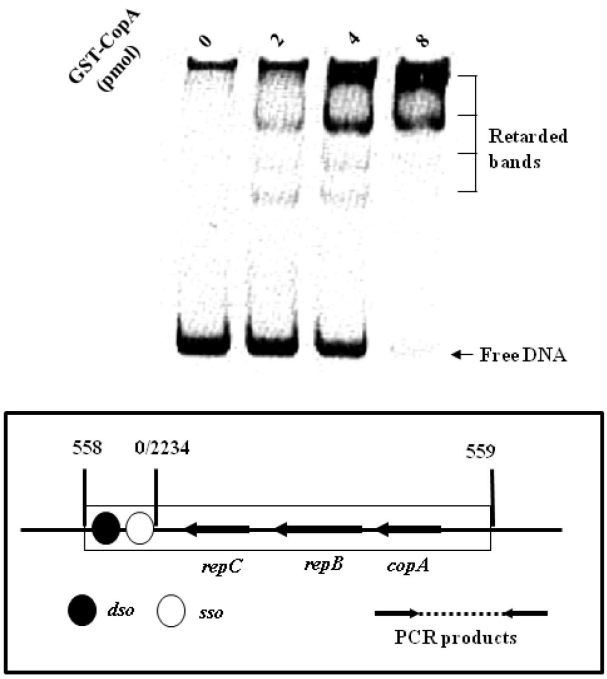

Fig. 4. Gel retardation assays. The 410 bp PCR products containing a putative operator of pJB01 *repABC* operon were incubated with GST-CopA proteins. Free DNA indicates PCR products containing *repABC* putative operator as shown by PCR products in lower panel. Retarded bands indicate the PCR products mobility-shifted by interaction with GST-CopA.

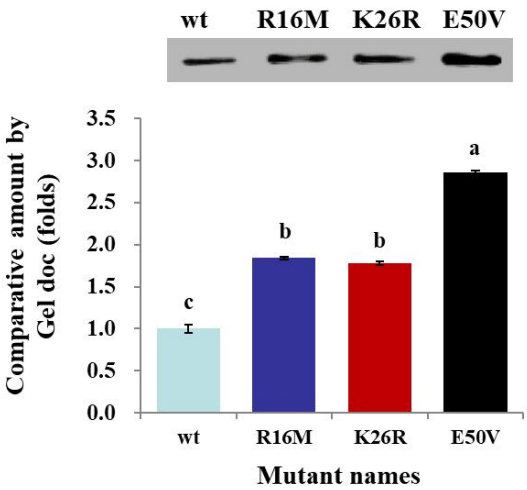

Fig. 5. Amounts of expressed mRNA from pJB01 derivatives containing wt and mutated *copA* genes through RT-PCR. Total RNA was isolated by RNeasy Mini kit (Qiagen). Syntheses of cDNA were done by using superscript II RTase (Gibco-BRL) according to manufacture's directions and then amplified by general PCR. X-axis indicates wt and mutant names, and Y-axis indicates ratios of expression level between wt CopA and each mutant.

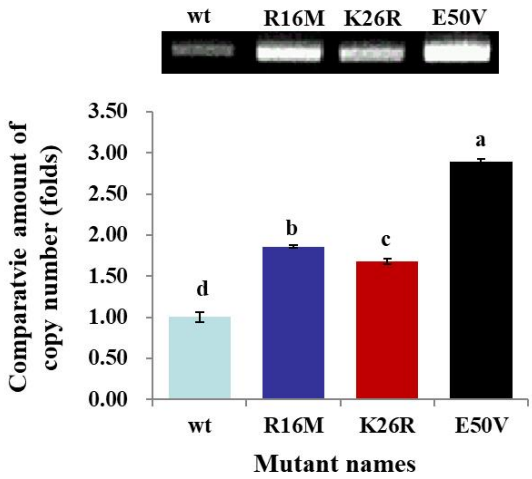

Fig. 6. Copy number analyses of wt CopA and its mutants. The copy number was examined by amount of covalently closed circular monomer plasmid at mid-exponential phase. An upper panel exhibits gel image for analyses of copy numbers of pJB01 *ermC* by CopA mutants, R16M, K26R and E50V. The lower panel is converted to a bar chart by measuring densities of each band on gel image. X-axis indicates names of wt and mutants, and Y-axis indicates ratios of copy number of pJB01 *ermC* wt and *copA* mutants.

decrease binding affinity of operator with those mutants, and the decreased bindings with the operator result in the increased expressions of repABC transcript depending on the mutants.

In order to examine changes of copy number owing to mutations of *copA* gene, their copy numbers were examined in *E. faecium* JS2. As shown in Fig. 6, copy numbers of *copA* mutants, R16M, K26R and E50V, on pJB01 *ermC* were increased to 1.86, 1.68 and 2.89 folds more than that of *copA* wt, respectively. From these results, it was suggested that CopA regulates the copy number by reducing transcriptional expression of RepB.

In summary, CopA binds operator of *repABC* operon and regulates expression of *repABC* gene on transcriptional level.

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

References

1. Acebo, P., Alda, M. T., Espinosa, M. and del Solar, G. 1996. Isolation and characterization of pLS1 plasmid mutants with increased copy numbers. *FEMS Microbiol. Lett.*

140, 85-91.
2. Acebo, P., de Lacoba, M. G., Rivas, G., Andreu, J. M. and Espinosa, M. 1998. Structural features of the plasmid pMV158-encoded transcriptional repressor CopG, a protein sharing similarities with both helix-turn-helix and β-sheet DNA binding proteins. *Proteins: Struct. Funct. Genet.* **32**, 248-261.
3. Brantl, S. and Wagner, E. G. H. 1996. An unusually long-lived antisense RNA in plasmid copy number control: *in vivo* RNAs encoded by the streptococcal plasmid pIP501. *J. Mol. Biol.* **255**, 275-288.
4. Chapkauskaitse, E., Bos, J., Sentkowska, D., Wawrzyniak, P., Wyszyńska, A., Szuplewska, M. and Bartosik, D. 2022. Differential localization and functional specialization of parS centromere-like sites in *repABC* replicons of Alphaproteobacteria. *Czarnecki. J. Appl. Environ. Microbiol*. **88**, e0020722.
5. Chattoraj, D. K. 2000. Control of plasmid DNA replication by iterons: no longer paradoxical. *Mol. Microbiol*. **37**, 467-476.
6. Costa, M., Sola, M., del Solar, G., Eritja, R., Hernandez-Arriaga, A. M., Espinosa, M., Gomis-Ruth, F. X. and Coll, M. 2001. Plasmid transcriptional repressor CopG oligomerises to render helical superstructures unbound and in complexes with oligonucleotides. *J. Mol. Biol*. **310**, 403-417.
7. del Solar, G., Acebo, P. and Espinosa, M. 1995. Replication control of plasmid pLS1: efficient regulation of plasmid copy number is exerted by the combined action of two plasmid components, CopG and RNA II. *Mol. Microbiol.* **18**, 913-924.
8. del Solar, G., Acebo, P. and Espinosa, M. 1995. Replication control of plasmid pLS1: the antisense RNA II and the compact *rnaII* region are involved in translational regulation of the initiator RepB synthesis. *Mol. Microbiol*. **23**, 95-108.
9. del Solar, G., Acebo, P. and Espinosa, M. 1997. Replication control of plasmid pLS1: the antisense *RNAII* and the compact *rnaII* region are involved in translational regulation of the initiator RepB synthesis. *Mol. Microbiol*. **23**, 95-108.
10. del Solar, G., de la Campa, A. G., Perez-Martin, J. P., Choli, T. and Espinosa, M. 1989. Purification and characterization of RepA, a protein involved in the copy number control of plasmid pLS1. *Nucleic Acids Res*. **17**, 2405-2420.
11. del Solar, G. and Espinosa, M. 1992. The copy number of plasmid pLS1 is regulated by two trans-acting plasmid products: the antisense RNA II and the repressor protein, RepA. *Mol. Microbiol*. **6**, 83-94.
12. del Solar, G. and Espinosa, M. 2000. Plasmid copy number control: an ever-growing story. *Mol. Microbiol*. **37**, 492-500.
13. del Solar, G., Giraldo, R., Ruiz-Echevarria, M. J. and Diaz-Orejas, R. 1998. Replication and control of circular bacterial plasmid. Microbiol. *Mol. Biol. Reviews* **62**, 434-464
14. Espinosa, M., del Solar, G., Rojo, F. and Alonso, J. C. 1995. Plasmid rolling-circle replication and its control. *FEMS microbial. Lett*. **130**, 111-120.
15. Garcillan-Barcia, M. P., Pluta, R, Lorenzo-Díaz, F., Bravo, A. and Espinosa, M. 2022. The facts and family secrets of plasmids that replicate via the rolling-circle mechanism. *Microbiol. Mol. Biol. Rev.* **86**, e0022220.
16. Gomis-Ruth, F. X., Sola, M., Acebo, P., Parraga, A., Guasch, A., Eritja, R., Gonzalez, A., Espinosa, M., del Solar, G. and Coll, M. 1998a. The structure of plasmid-encoded transcriptional repressor CopG unliganded and bound to its operator. *EMBO J*. **17**, 7404-7415.
17. Gomis-Ruth, F. X., Sola, M., Perez-Luque, R., Acebo, P., Alda, M. T., Gonzalez, A., Espinosa, M., del Solar, G. and Coll, M, 1998b. Overexpression, purification, crystallization and preliminary X-ray diffraction analysis of the pMV158-encoded plasmid transcriptional repressor protein CopG. *FEBS Letters* **425**, 161-165.
18. Khan, S. A. 1996. Mechanism of replication and copy number control of plasmids in Gram-positive bacteria. *Genet. Eng.* **18**, 183-201.
19. Kim, S. W., Jeong, I. S., Jeong, E. J., Tak, J. I., Lee, J. H., Eo, S. K., Kang, H. Y. and Bahk, J. D. 2008. The terminal and internal hairpin loops of the ctRNA of plasmid pJB01 play critical roles in regulating copy number. *Mol. Cells* **26**, 26-33.
20. Kim, S. W., Jeong, E. J., Kang, H. S., Tak, J. I., Bang, W. Y., Heo, J. B., Jeong, J. Y., Yoon, G. M., Kang, H.Y. and Bahk, J. D. 2006. Role of RepB in the replication of plasmid pJB01 isolated from *Enterococcus faecium* JC1. *Plasmid* **55**, 99-113.
21. Kim, S. W., Kang, H. Y., Gal, S. W., Cho, K. K. and Bahk, J. D. 2011. RepC as a negative copy number regulator is involved in the maintenance of pJB01 homeostasis. *Afr. J. Microbiol. Res*. **5**, 2583-2589.
22. McEachem, M. J., Bott, M. A., Tooker, P. A. and Heliski, D. R. 1989. Negative control of plasmid R6K replication: possible role of intermolecular coupling of replication origins. *Proc. Natl. Acad. Sci. USA*. **86**, 7942-7946.
23. Mcpherson, M. J., Quirke, P. and Taylor, G. R. 1993. PCR, A practical Approach. IRL Press, 207–209.
24. Nordstrom, K., Molin, S. and Light, J. 1984. Control of replication of bacterial plasmids: genetics, molecular biology, and physiology of the plasmid R1 system. *Plasmid* **12**, 71-90.
25. Pal, S. K. and Chattoraj, D. K. 1988. P1 plasmid replication: initiator sequestration is inadequate to explain control by initiator-binding sites. *J. Bacteriol*. **170**, 3554-3560.
26. Ruiz-Maso, J. A., Luengo, L. M., Moreno-Cordoba, I., Diaz-Orejas, R., Del Solar, G. 2017. Successful Establishment of plasmids R1 and pMV158 in a new host requires the relief of the transcriptional repression of their essential rep genes. *Front. Microbiol*. **8**, 2367.
27. Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning, A laboratory manual. 2nd edition, Cold Spring

Harbor Laboratory. Cold Spring Harbor, NY, 1.25-28, 1. 38-41.
28. van der Vossen, J. M., Kok, J. and Venema, G. 1985. Construction of cloning, promoter-screening, and terminator-screening shuttle vectors for *Bacillus subtilis* and *Streptococcus lactis*. *Appl. Environ. Microbiol*. **50**, 540-542.
29. Wanger, E. G. H. and Simons, R. W. 1994. Antisense RNA control in bacteria, phages, and plasmids, *Annu. Rev. Microbiol*. **48**, 713-742.
30. Zhang, H., Hao, Y., Zhang, D. and Luo, Y. 2011. Characterization of the cryptic plasmid pTXW from *Lactobacillus paracasei* TXW. *Plasmid* **65**, 1-7.

초록 : 전사 수준에서 *repABC* 유전자 발현을 조절하는 CopA 단백질의 역할

김삼웅[1] · 갈상완[2] · 지원재[3] · 방우영[4] · 김태완[5] · 백인규[6] · 방규호[2]*
([1]경상국립대학교 농식품바이오융복합연구원, [2]경상국립대학교 제약공학과, [3]국립생물자원관 생물다양성연구과, [4]국립생물자원관 생물다양성총괄과, [5]경상국립대학교 산학연협력 선도대학 육성사업단, [6]경상국립대학교 수생동물질병연구실)

플라스미드의 복제는 엄격하게 조절되어야 하기 때문에 일반적으로 rolling circle 복제를 수행하는 플라스미드들은 복제 개시인자인 RepB는 전사 및 번역 수준에서 엄격하게 조절되어 일정한 copy number를 유지한다. 플라스미드 pJB01에는 단일 오페론으로 구성된 세 개의 *orf*s (*copA*, *repB*, *repC* 또는 *repABC*)가 포함되어 있다. 아미노산 서열 분석에서 pJB01 CopA는 다른 플라스미드의 복제 수 조절 단백질로서 Cops와 상동성을 보였다. pMV158의 CopG와 비교할 때, CopA는 플라스미드의 일반화된 억제자의 모티브로 알려진 RHH (ribbon-helix-helix)를 형성하는 것으로 추정된다. gel mobility shift assay 결과 정제된 융합 단백질이 *repABC* 오페론의 operator 영역에 결합하는 것으로 나타났다. 전사 수준에 대한 CopA의 기능적 역할을 조사하기 위해 CopA R16M, K26R 및 E50V와 같은 세 개의 포인트 돌연변이가 CopA의 코딩 프레임에서 구성되었다. CopA R16M, K26R 및 E50V 돌연변이의 repABC mRNA 수준은 CopA wt보다 각각 1.84, 1.78 및 2.86배 증가했다. 또한 세 개의 CopA 유전자의 돌연변이로 인한 복제 수도 CopA wt보다 각각 1.86, 1.68 및 2.89배 증가했다. 이러한 결과는 CopA가 전사 억제자이며 복제 개시자로서 repABC mRNA 및 RepB 단백질 수를 감소시킴으로써 pJB01의 복제 수를 감소시키는 것으로 제의된다.