

# The genetic switch for the regulatory pathway of Lactobacillus plantarum phage $\phi$ gle

メタデータ	言語: eng 出版者: 公開日: 2017-10-05 キーワード (Ja): キーワード (En): 作成者: メールアドレス: 所属:
URL	<a href="http://hdl.handle.net/2297/1090">http://hdl.handle.net/2297/1090</a>

# The genetic switch for the regulatory pathway of *Lactobacillus plantarum* phage $\phi$ g1e

Makiko KAKIKAWA<sup>1</sup>, Shingo OHKUBO<sup>2</sup>, Michio SAYAMA<sup>2</sup>, Akira TAKETO<sup>3</sup>, Ken-Ichi KODAIRA<sup>2</sup>

<sup>1</sup>Faculty of Engineering, Kanazawa University, 2-40-20, Kodatsuno, Kanazawa, 920-8667, Japan

<sup>2</sup>Molecular Biology Group, Faculty of Engineering, Toyama University, 3190, Gofuku, Toyama, 930-8555, Japan

<sup>3</sup>Department of Applied physics and Chemistry, Fukui University of Technology, Gakuen, Fukui 910-8505, Japan

Prevalence of lysogeny in various Gram-positive and -negative bacteria has been reported. For their propagation in two different ways (lysis and lysogeny), it has been thought that the temperate phages have a set of regulatory proteins, as well their recognizable DNA domains. And the temperate phages have been regarded as valuable genetic tools for gene transfer, expression and cloning, in these industrially and medically important genera.

In *Escherichia coli* phages such as lamda, P2 and 186, the lysis-lysogeny decision depends upon phage-encoded proteins, known as repression by binding to specific DNA sequences called operators. Contrary to the coliphage, molecular details on the lysis-lysogeny switch of the phages from lactic acid bacteria are scanty, although several putative repressor/operator systems have been reported.

The *Lactobacillus plantarum* phage  $\phi$ g1e (42 259 bp) has two repressor-like genes *cpg* and *cng* oriented oppositely, accompanied by two potential promoters  $P_R$ ,  $P_L$  and seven operator-like 15 bp sequences (GATAC-Boxes). Box 1 (Gb1) is located within *cng*, the five boxes 2-6 (Gb2- Gb6) are between *cng* and *cpg*, and box 7 (Gb7) is downstream of *cpg* (Fig. 1.).

In this study, several domains within the  $P_L$ /Gb5- Gb6- *cpg*-Gb7,  $\sim$ 530 bp region were cloned upstream of *cat* of plasmid pKK232-8, and the promoter / repressor abilities were examined.

*E. coli* XL1-Blue carrying pKPL1 (*cat* under  $P_L$ /Gb2-Gb6, 454 bp) or pKPL2 (*cat* under  $P_L$ /Gb5-Gb6, 122 bp) multiplied in the presence of up to 200  $\mu$ g/ml of chloramphenicol (Cm), whereas XL1-Blue / pKK232-8 did not

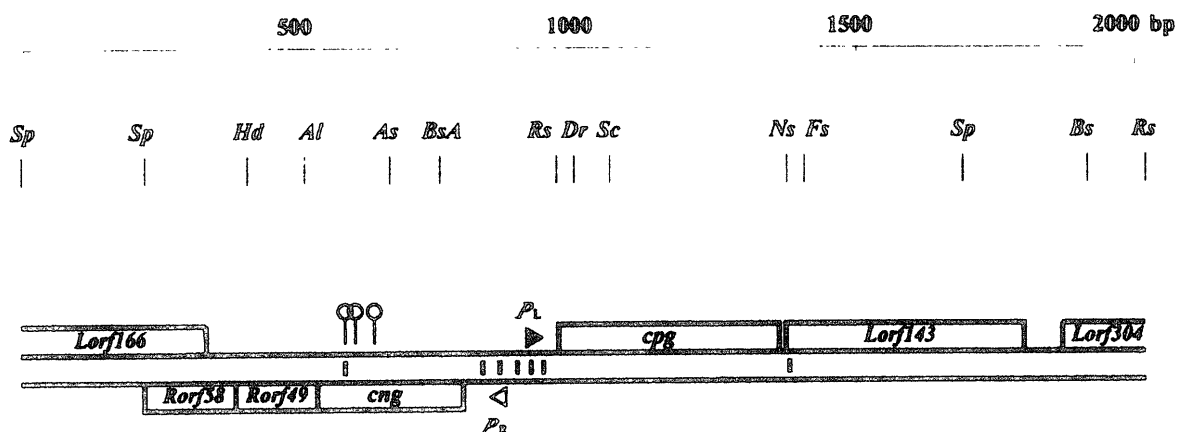


Fig. 1. A 2030 bp physical map of the  $\phi$ g1e genome.

$\phi$ g1e genome is shown by two thick lines. The two promoters are indicated by  $P_L$  (closed arrowhead) and  $P_R$  (open arrowhead) above or below the  $\phi$ g1e DNA depending on their direction. The genes *cng*, *cpg* and putative orfs are indicated by open boxes with their directions. The seven GATAC-boxes are indicated by small closed boxes.

CAT activity of *E. coli* XL1-Blue carrying  $\phi$ gIe promoter-containing plasmids<sup>a</sup>

Plasmids <sup>d</sup>	Promoter	CAT activity <sup>b</sup> with <sup>c</sup>	
		No plasmids	pAPLCP1
pKK232-8	None	<0.5	<0.5
pKPL1	$P_R-P_L$	49.6	- <sup>e</sup>
pKPL2	$P_L$	41.8	4.7
pKRL3	$P_L-P_L$	96.2	12.0
pKPLCP1	$P_L$ <i>cpg</i>	3.7	- <sup>e</sup>
pKPR1	$P_R$	1090.0	31.0

<sup>a</sup> CAT activity was assayed as described by Kakikawa et al. (1998).

<sup>b</sup> Values, average of several (five to six) experiments.

<sup>c</sup> Plasmid pAPLCP1, co-resident with promoter - containing plasmid.

<sup>d</sup> Plasmids containing  $\phi$ gIe promoters ( $P_R-P_L$ ,  $P_L$  and  $P_L-P_R$ ) upstream of *cat* are indicated in Fig. 1. pKPR1 containing  $P_R$ -*cat* from Kakikawa et al.(1998).

<sup>e</sup> Not done.

grow in the presence of 5  $\mu$ g/ml of Cm. Promoter activity of pKPL2 (*cat* under  $P_L$ /Gb5-Gb6) was estimated by CAT assay in *E. coli*. The mean value of pKPR2 was 41.8 units /mg, whereas that of pKK232-8 (control) was 0.5 unit /mg. These results indicate that 122 bp region of  $P_L$  /Gb5-Gb6, function as a promoter.

The activity of pKPLCP1 (*cat* under  $P_L$ /Gb5-Gb6-*cpg*, 478 bp) was only marginal (3.7 units /mg). When pKPL2 was coexistent with a compatible derivative of plasmid pACYC177 carrying  $P_L$ /Gb5-Gb6-*cpg* (478 bp), the CAT activity was declined to the level of pKPLCP1. These results suggest that *cpg* functions as a repressor of transcription from  $P_L$ .

On the other hand, the *cpg*-encoded protein Cpg was overproduced in *E. coli* under  $P_{T7}$ . The molecular mass of the purified Cpg (14.5 kDa on a SDS gel) corresponded well with that (15.1 kDa) predicted from the DNA sequence. Gel-shift and footprinting assays demonstrated that Cpg selectively binds to about 25 bp bases centered on the GATAC-box (from 1 to7). In seven GATAC-Box, Cpg seemed to bind initially to Gb4 located between  $P_L$  and  $P_R$ . Moreover, protein crosslinking experiments using glutaraldehyde showed that Cpg most likely functions as a dimeric form.

Thus, the present results indicate that Cpg probably repress  $P_L$  through binding to the operator GATAC-box (es), and the  $P_L$ /*cpg* region might participate in the lysogenic pathway.

Cpg ( $\mu$ g)	-	0.1	0.2	0.2	0.2	0.2	0.2
Self (nM)	-	-	-	12.5	25.0	-	-
Nonself (nM)	-	-	-	-	-	12.5	25.0



Fig. 2 Gel-Shift assays.

A  $P^{32}$ -labelled DNA fragment (0.5 nM) containing Gb7 was mixed with purified Cpg in 20  $\mu$ l of reaction buffer [ 10 mM Tris-HCl (pH 7.8), 150 mM KCl, 1 mM EDTA, 0.1 mM DTT, 5% glycerol, 50.0g/ml BSA] with or without competitor (self or non-self) as indicated. After incubation at 30. for 10 min, the mixture was electrophoresed on a non-denaturing polyacrylamide gel at 4., and the DNA bands were visualized by autoradiography.