The genetic switch for the regulatory pathway of Lactobacillus plantarum phage ϕg1e

The genetic switch for the regulatory pathway of *Lactobacillus plantarum* phage φg1e

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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 *Esherichia 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 φg1e (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, ~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 u p to 200 g/ml of chloramphenicol (Cm), whereas XL1-Blue / pKK232-8 did not

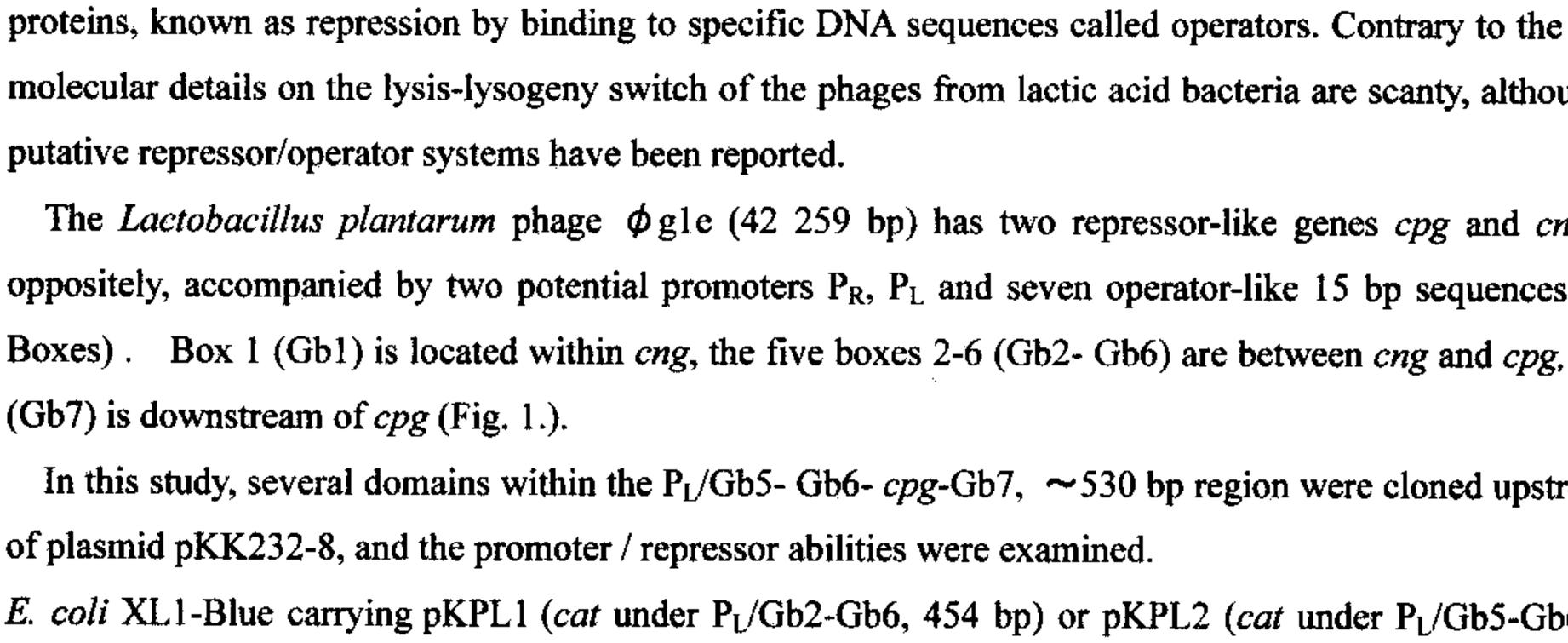

Fig. 1. A 2030 bp physical map of the φg1e genome .

φg1e 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 φg1e 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 φg1e promoter-containing plasmids[a]

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

[a] CAT activity was assayed as described by Kakikawa et al. (1998).

[b] Values, average of several (five to six) experiments.

[c] Plasmid pAPLCP1, co-resident with promoter - containing plasmid.

[d] Plasmids containing φg1epromoters (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).

[e] Not done.

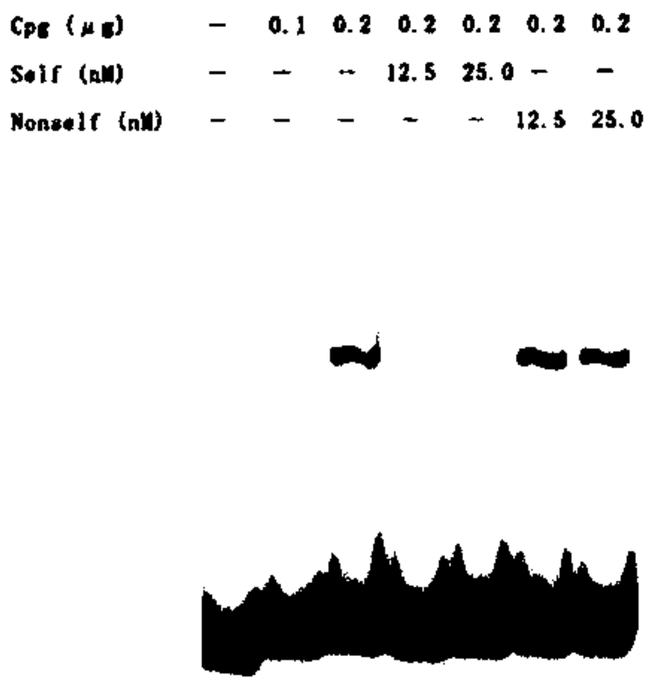

Fig. 2 Gel-Shift assays.

A P^{32}-labelled DNA fragment (0.5 nM) containing Gb7 was mixed with purified Cpg in 20 .l of reaction buffer [10 mM Tris-HCl (pH 7.8), 150 mM KCl, 1 mM EDTA, 0.1 mM DTT, 5% glycerol, 50 g/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.

grow in the presence of 5 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.