Short Communication

Construction of a Promoter-cloning Vector in *Zymomonas mobilis*Hideshi YANASE, Jun KURII
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Received July 16, 1986

Zymomonas mobilis is a gram-negative bacterium capable of producing almost the theoretical yield of ethanol from glucose and showing higher productivity than industrial yeasts.¹⁾ This organism is potentially useful for the commercial production of ethanol. However, it has a limited substrate range and can only utilize glucose, fructose and sucrose.²⁾ To use *Z. mobilis* for industrial ethanol production, it is necessary to improve it by means of recombinant DNA techniques. Therefore, we have developed fundamental genetic methods; the spheroplast fusion of *Z. mobilis*,³⁾ the construction of shuttle vectors between *Z. mobilis* and *Escherichia coli*,⁴⁾ and the establishment of a transformation method for *Z. mobilis*.⁵⁾ Using these techniques, we are attempting to introduce an *E. coli* gene into *Z. mobilis*. However, for full expression of the gene, information on the structure and function of a promoter region of *Zymomonas* have been required. The present paper deals with the construction of a promoter-cloning vector in *Z. mobilis* using a *lac* operon from pMC1403.⁶⁾

The bacterial strains used in this experiment were *E. coli* MC1061 (*Alac*, *Sm*^r, *rec*⁻) and *Z. mobilis* var. *recifensis* NRRL B-14023 (designated as *Z. mobilis* CP4). The plasmids used were pMC1403 (*Alac*, *Ap*^r: lacks the promoter region and the first eight codons of the N-

terminal end of the β -galactosidase gene)⁶⁾ and pZA32 (*Cm*^r: a shuttle vector between *Z. mobilis* and *E. coli*, which was constructed by inserting the 3.9-kb cryptic plasmid of *Z. mobilis* ATCC10988 into *E. coli* vector pACYC184).⁴⁾ *E. coli* was grown in L-broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.1% glucose, pH 7.2) and *Z. mobilis* in RM medium (2% glucose, 1% yeast extract, 0.2% KH_2PO_4 , pH 6.0). *E. coli* was transformed with plasmid DNA by the method of Mandel and Higa,⁷⁾ and *Z. mobilis* by a conjugational method using a helper plasmid, pRK2013, or by means of the partial spheroplast transformation described in the previous papers.⁵⁾ For screening of a *Lac*⁺ clone of *E. coli*, lactose-MacConkey medium (1.7% peptone, 0.3% proteose peptone, 1% lactose, 0.15% sodium cholate, 0.5% NaCl, 3 mg% neutral red, 0.1 mg% crystal violet, 1.35% agar, pH 7.1) was used, and for *Zymomonas* RM medium containing 50 $\mu\text{g}/\text{ml}$ of X-gal was used. β -Galactosidase was assayed by measuring *o*-nitrophenol liberated from ONPG.⁸⁾

The promoter-cloning vector, pZL1, was constructed as shown in Fig. 1. The *Bam*HI-*Sal*I-digested fragment of the lactose operon in pMC1403 was ligated with the large fragment of *Bam*HI-*Sal*I-digested pZA32 to construct a recombinant plasmid, pZL1, which was then introduced into *E. coli* MC1061. It was 13.8 kb in size, carried *Cm*^r and could transform *Z. mobilis* as well as *E. coli*.

For the screening of a functional promoter DNA fragment of *Z. mobilis* in *E. coli* MC1061, the chromosomal DNA of *Z. mobilis* CP4 was cleaved with *Bam*HI and then ligated with the *Bam*HI-cleaved pZL1 plasmid DNA. The recombinant DNA thus obtained transformed *E. coli* MC1061. *Lac*⁺ colonies in which promoter fragments were cloned into pZL1 could be selected on a lactose-MacConkey indicator agar plate containing 20 $\mu\text{g}/\text{ml}$ of *Cm*. Plasmid DNAs were extracted from six out of twenty *Lac*⁺ clones, and

FIG. 1. Construction of a Promoter-cloning Vector, pZL1.

lac genes Z and Y code for β -galactosidase and galactoside permease, respectively. *lac'*Z indicates that the lactose operon lacks the promoter region and the first eight codons of the N-terminal end of the β -galactosidase gene.

introduced into *Z. mobilis* CP4 by the conjugational method or by means of partial spheroplast transformation. Lac⁺ *Z. mobilis* was selected on an RM agar plate containing 50 μ g/ml of X-gal and 100 μ g/ml of Cm as a blue colony.

To determine the activity of the promoter, the β -galactosidase activity of Lac⁺ clones was assayed in *E. coli* and *Z. mobilis*, as summarized in Table I. The clones were divided into three types: in Type 1, the β -galactosidase activity of *E. coli* was higher than that in *Z. mobilis*, in spite of the promoter DNA arising from *Z. mobilis* (pZL2 and pZL4); in Type 2, the level of β -galactosidase activity in *Z. mobilis* was almost the same as that of *E. coli* (pZL3, pZL6 and pZL7); and in Type 3, the β -galactosidase activity in *Z. mobilis* was re-

markably higher than that in *E. coli*, and much higher than the level in *E. coli* fully induced by IPTG (pZL5). Lac⁺ plasmids were extracted from *E. coli*, cleaved with *Bam*HI and then analyzed by agarose gel electrophoresis. The size of the inserted DNA fragment was measured and found to be between 1.6 and 10.9 kb in size.

From the results presented here, it was found that pZL1 was a potential promoter-cloning vector for *Z. mobilis*, and that the *lac* operon of *E. coli* was expressed in *Z. mobilis*. The production of ethanol from lactose and whey by *Z. mobilis* cells harbouring the Lac⁺ recombinant plasmid will be reported elsewhere.

TABLE I. EXPRESSION OF THE β -GALACTOSIDASE GENE ON *lac*⁺Z, Y IN *E. coli* MC1061 AND *Z. mobilis* CP4

E. coli and *Z. mobilis* were grown in L-broth and RM medium, respectively, at 30°C for 24 hr. The cells were treated with a one-twentieth volume of toluene for 15 to 30 sec prior to the β -galactosidase assay. The β -galactosidase activity was determined in a reaction mixture containing toluenized cells (about 15 μ g of protein), 0.5 mg of ONPG, 100 μ mol of sodium phosphate buffer (pH 7.5), 100 μ mol of 2-mercaptoethanol and 1 μ mol of MgSO₄·7H₂O in a final volume of 1.0 ml. The reaction was carried out at 28°C for 10 min, and stopped with 1.0 ml of 0.1 M Na₂CO₃. The increase in absorbance at 420 nm was measured. One unit (U) of β -galactosidase was taken as the amount of the enzyme which liberated 1 nmol of *o*-nitrophenol per min. The size of DNA fragments was determined by agarose gel electrophoresis using λ phage DNA digested with *Hind*III as a standard.

Plasmid	Size (kb) of <i>Z. mobilis</i> DNA inserted	β -Galactosidase (U/mg protein)		B/A ^a
		in <i>E. coli</i> (A)	in <i>Z. mobilis</i> (B)	
None	—	2	2	—
pZL1	—	2	2	—
pZL2	1.6	1944	449	0.2
pZL3	6.0	145	140	1.0
pZL4	1.6	1574	385	0.2
pZL5	10.9	305	5864	19
pZL6	2.4	994	578	0.6
pZL7	2.4	1004	823	0.8
<i>E. coli</i> K12 ^b				
(-IPTG)		70		
(+IPTG)		4440		

^a B/A, Ratio of β -galactosidase activity in *Z. mobilis* to that in *E. coli*.

^b A Lac⁺ strain of *E. coli* K12 was grown with or without 1 mM IPTG.

Acknowledgment. This work was supported in part by a Grant-in Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

- 1) P. L. Rogers, K. J. Lee and D. E. Tribe, *Biotechnol. Lett.*, **1**, 165 (1979).
- 2) J. Swings and J. D. Ley, *Bacteriol. Rev.*, **41**, 1 (1977).
- 3) H. Yanase, M. Yasui, T. Miyazaki and K. Tonomura, *Agric. Biol. Chem.*, **49**, 133 (1985).
- 4) K. Tonomura, T. Okamoto, M. Yasui and H. Yanase, *Agric. Biol. Chem.*, **50**, 805 (1986).
- 5) H. Yanase, T. Kotani and K. Tonomura, *Agric. Biol. Chem.*, **50**, in press (1986).
- 6) M. J. Casadaban, J. Chou and S. N. Cohen, *J. Bacteriol.*, **143**, 1971 (1980).
- 7) M. Mandel and A. Higa, *J. Mol. Biol.*, **53**, 154 (1970).
- 8) "Practical Methods in Molecular Biology," ed. by R. F. Schleif and P. C. Wensink, Springer-Verlag, New York, 1981, p. 43.