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Characterization of the 5′-Flanking Region Upstream from the Structural Gene for *Zymomonas mobilis* Alcohol Dehydrogenase

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A *Zymomonas mobilis* DNA fragment consisting of 207 nucleotides, which corresponded to the 5′-flanking region of an *adhB* gene encoding alcohol dehydrogenase II, was fused to the structural gene coding for a *Bacillus* endo-β-1,4-glucanase. The *Z. mobilis* DNA fragment was identified to promote 50-fold increase in the expression of endo-β-1,4-glucanase gene in *Escherichia coli*.

Key words: *Zymomonas mobilis*, promoter, alcohol dehydrogenase, endo-β-1,4-glucanase

Z. mobilis is a facultative anaerobic Gram-negative bacterium which can convert glucose to ethanol more rapidly and efficiently than yeast (5). Two isoenzymes of alcohol dehydrogenase (ADH) responsible for the final step of the alcoholic fermentation have been identified in *Z. mobilis*. They represent 2 to 5% of the soluble cell proteins in the organism. The ADHII is the dominant enzyme during fermentation, although both isoenzymes are expressed in *Z. mobilis* (3). The *adhB* genes encoding *Z. mobilis* ADHII have been cloned and sequenced from two strains of *Z. mobilis* (1, 6). The sites for transcriptional initiation of *adhB* gene of *Z. mobilis* ZM4 were identified by Conway *et al* (1). In this work, we observed that the 5′-flanking region upstream from the ATG start codon of *adhB* gene derived from *Z. mobilis* ATCC 10988 works efficiently as a promoter in *E. coli* by using a *Bacillus* endo-β-1,4-glucanase structural gene as a reporter.

Fusion of the putative promoter region of *adhB* into a endo-β-1,4-glucanase gene.

To observe whether the 5′-flanking region upstream from the ATG start codon of *adhB* gene (see Fig. 1 for nucleotide sequence) works as a promoter in *E. coli*, we used a *Bacillus* endo-β-1,4-glucanase structural gene as a reporter gene. We made two recombinant plasmids in which the putative promoter region of *adhB* gene was juxtaposed with 11 bp upstream from ATG start codon of the endo-β-1,4-glucanse gene. For the construction of recombinant plasmids, an *Eco*RI and *Alu*I-generated 215-bp DNA fragment corresponding to the 5′-flanking region of *adhB* gene was isolated from pADS98, which is a pUC9 derivative containing *adhB* gene of *Z. mobilis* ATCC 10988. Its *Eco*RI-generated sticky end was made blunt using Klenow fragment. This 215-bp fragment was inserted 11 bp upstream from ATG start codon of endoglucanase gene in *Sma*I-digested pUBS102-9 (4), a pUC9 derivative carrying the *Bacillus* endo-β-1,4-glucanase gene without its own promoter and SD sequence (Fig. 2). Two plasmid constructs were obtained from the ampicillin-resistant *E. coli* transformants according to the orientation of the 215-bp inserts with respect to the endoglucanase gene, and named pAPC207 and pAPC700, respectively, as described in Fig. 2.

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      -200
GATCTGATAAAACTGATAGACATATTGCTTTTGCGCTGCCCGATTGCTGAAAATGCGTAAAATTGGTGATTTTAC

                  -100
TCGTTTTCAGGAAAAACTTTGAGAAAACGTCTCGAAAACGGGATTAAAACGCAAAAACAATAGAAAGCGATTTCG

                                                  -1
CGAAAATGGTTGTTTTCGGGTTGTTGCTTTAAACTAGTATGTAGGGTGAGGTTATAGCT
                                  S.D.          ↑
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Fig. 1. The nucleotide sequence of 5′-flanking region upstream from the ATG translational start codon of *adhB* gene for the *Z. mobilis* ADH. A putative ribosomal binding site was underlined. The arrow indicated the cleavage site for restriction enzyme *Alu*I. The number above the sequence indicated the number of nucleotide from the A in the translation initiation ATG. The sequence data have been submitted to the EMBL nucleotide sequence data base under accession number X17065.

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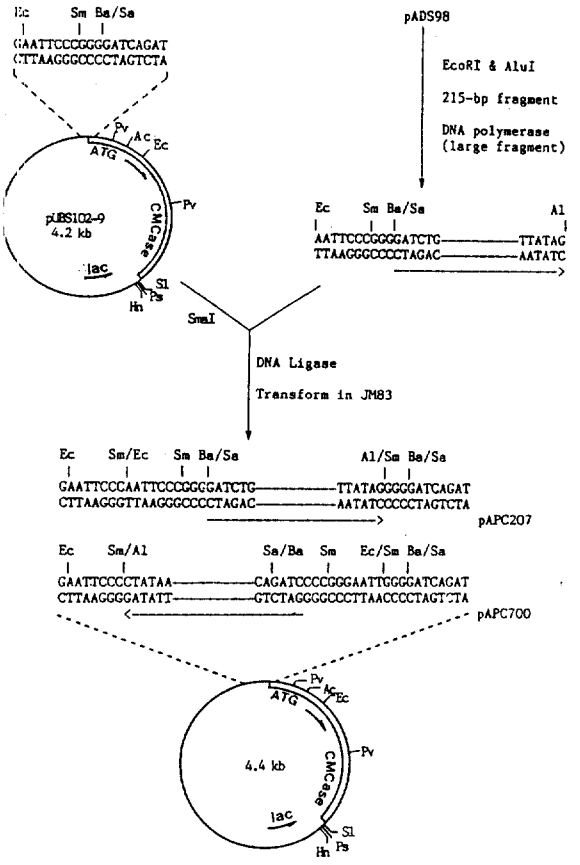

Fig. 2. Strategy used to express a *Bacillus* endo-β-1,4-glucanase gene by the 5′-flanking region of *adhB* gene. The 207-bp fragment corresponding to −3 to −209 positions in 5′-flanking region of *adhB* gene was underlined by the dashed line, with the arrow indicating the direction of transcription for *adhB* gene. With its ATG start codon the endo-β-1,4-glucanase gene on plasmid pUBS 102-9 was shown as solid bar. The arrow on the plasmid indicated the direction of transcription for the endo-β-1,4-glucanase gene. Restriction enzyme cleavage sites were *Eco*RI (Ec), *Sau*3AI (Sa), *Alu*I (Al), *Sma*I (Sm), *Pst*I (Ps), *Acc*I (Ac), *Pvu*II (Pv), *Sal*I (Sl), *Bam*HI (Ba), and *Hin*dIII (Hn).

Expression of the endo-β-1,4-glucanase gene

In order to measure the level of expression of the endoglucanase gene, the endoglucanase activities of cell-free extracts from *E. coli* cells harboring the recombinant plasmids were assayed by DNS method (2). The results are summarized in Table 1. The endo-β-1,4-glucanase gene on the plasmid pAPC207 was expressed at a level 50 times greater than that on pUC109-2 only when the 5′-flanking region of *adhB* was oriented into the direction of transcription for endoglucanase gene as that of *adhB* gene. However, the expression level of the gene on plasmid pAPC700 could be comparable to that of the gene on pUC109-2 when the 5′-flanking region was located opposite to the direction of transcription for endoglucanase gene. These results indicate that the 5′-flanking region of the *Z. mobilis adhB* gene had been efficiently used to express a foreign gene at the transcriptional and translational levels in *E. coli*.

Table 1. Endo-β-1,4-glucanase activities of *E. coli* cells transformed with the plasmids

Plasmids	Endo-β-1,4-glucanase activity[a] (mU/mg protein)
pUC9	ND[b]
pUBS102-9	40
pAPC207	1,870
pAPC700	50

[a] Endo-β-1,4-glucanase activities were determined by DNS method after growing cell overnight on LB broth consisting of yeast extract (5 g/liter), bacto-tryptone (10 g/liter) and NaCl (5 g/liter). Each values represents the average of four determinants.
[b] ND, not detectable activity.

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