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Expression of Developmentally Regulated Promoter of Alkali-tolerant *Bacillus* sp. YA-14

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알칼리 내성 *Bacillus* sp. YA-14 에서 유래된 생육단계 조절 promoter 의 발현

박영서 · 구본탁 · 김진만 · 박희경 · 유주현*

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The promoter isolated from chromosomal DNA of an alkali-tolerant *Bacillus* sp. YA-14 was subcloned and biochemically characterized. Also the relationships between the promoter activity and sporulation were investigated. In alkali-tolerant *Bacillus* sp. and *Bacillus* subtilis, the activity of promoter began to increase at the onset of sporulation with the same mode, and repressed in the presence of 1.0% (w/v) glucose. Among five *spoO* genes, three *spoO* genes (*spoOB, spoH, spoOJ*) were required for promoter expression.

In response to nutrient depletion *Bacillus subtilis* initiates a complex program of temporal gene expression. Temporal gene expression in *B. subtilis* is accompanied by changes in the predominant forms of RNA polymerase within the cells (1-3).

The *B. subtilis* RNA polymerase is comprised of core RNA polymerase and a sigma factor of characteristic molecular weight (2). The sigma subunit of RNA polymerase is responsible for promoter selection, and the nucleotide sequence of promoters that respond to the various forms of RNA polymerase differ (3). Sporulation-specific forms of RNA polymerases are presumably involved in the recognition of specific promoter sequences that control the expression of genes whose products are needed during sporulation.

Using the promoter probe plasmid pPL703 (4), we have isolated a temporally regulated promoter from the alkali-tolerant *Bacillus* sp. chromosome, and its characteristics have been investigated (5-7).

In the current study, we subcloned the recombinant plasmid harboring promoter fragment and investigated the biochemical characteristics in *B. subtilis* and the doner strain. And the relationship between the expression of the promoter and the sporulation was also demonstrated.

key words: Promoter, *spoO* gene, alkali-tolerant *Bacillus* sp.
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Materials and Methods

Bacterial strains and plasmids

Bacterial strains and plasmids used in this study are listed in Table 1. *B. subtilis* 207-25 (8) was used as host for all the *B. subtilis* plasmids employed in this study. Alkali-tolerant *Bacillus* sp. YA-14 was isolated from soil (9) and used as donor strain of promoter. We have cloned previously the pectate lyase gene and the xylanase gene from the chromosomal DNA of this strain (10, 11). Promoter probe plasmid pPL703 contains a promoterless chloramphenicol acetyltransferase gene (*cat-86*) originated from *Bacillus pumilus*. Phenotypic expression of chloramphenicol resistance (Cm^r) gene from pPL703 in *B. subtilis* requires insertion of *Bacillus* promoters in front of the Cm^r gene. p-12B1 is pPL703 derivative that contains the 1.6 kb promoter fragment of *Bacillus* sp. YA-14 chromosome.

Table 1. List of bacterial strains and plasmids

Strain/Plasmid	Relevant properties	Reference
B. subtilis 207-25	*r⁻m⁻amyE*07 *hsrM aroI*906 *lys*21 *leuA*8 recE4	Tamura *et al.* (8)
B. subtilis JH642	*pheA*1 *trpC*2	BGSC
B. subtilis JH647	*pheA*1 *trpC*2 *spoOEII*	BGSC
B. subtilis JH648	*pheA*1 *trpC*2 *spoOB*136	BGSC
B. subtilis JH649	*pheA*1 *trpC*2 *spoOF*221	BGSC
B. subtilis JH651	*pheA*1 *trpC*2 *spoOH*81	BGSC
B. subtilis JH696	*pheA*1 *trpC*2 *spoOJ*87	BGSC
Bacillus sp. YA-14	alkali-tolerant strain	Yu *et al.* (9)
pPL703	promoter probe vector	Williams *et al.* (4)
p-12B1	pPL703 + 1.6 kb promoter from *Bacillus* sp. YA-14	Yu *et al.* (6)

BGSC: Bacillus Genetic Stock Center

Media

B. subtilis 207-25 was grown in PAB (Penassay broth, Difco) for protoplast transformation and the DM-3 medium (12) was used as regeneration medium. For the competent cell transformation of *Bacillus spo* mutant and the *Bacillus* sp. YA-14, SPI medium (2g $(NH_4)_2$ SO_4, 14g K_2HPO_4, 6g KH_2PO_4, 1g C_6H_6 Na_2O_3, 4g $MgSO_47H_2O$, 5g glucose, 1g yeast extract, 0.2g casamino acid, pH 8.0 per liter) was used (13). When the CAT activity was measured, the cells were grown in 2XSSG medium (14). Kanamycin and chloramphenicol were added in the media to the final concentration of 5 μg/m*l* and 10 μg/m*l*, respectively.

CAT enzyme assay

Promoter activity was expressed as CAT specific activity in lysates of cells. CAT was assayed by the colorimetric procedure (15) and the protein was measured by the Lowry method (16). The specific activity of CAT was expressed as μmol of chloramphenicol acetylated per min per mg of protein.

DNA manipulation and transformation

Minipreparation of *B. subtilis* plasmid DNA were carried out according to the method of Doi *et al.* (17). Standard procedures of Maniatis *et al* (18). were followed as described for other DNA manipulation.

Results and Discussions

Subcloning of p-12B1 promoter

Plasmid p-12B1 contains 1.6 kb *Bam*HI-*Pst*I *Bacillus* sp. YA-14 chromosomal DNA fragment showing promoter activity. In this DNA fragment, 0.7 kb *Sal*I-*Pst*I DNA fragment was not shown promoter activity (data not shown). p-12B1 was double digested with *Bam*HI and *Sal*I and the 0.9 kb DNA fragment was electroeluted and ligated with pPL703 digested with the same restriction endonucleases. *B. subtilis* 207-25 was transformed with ligated DNA and the recombinant DNA was isolated from the chloramphenicol resistant transformants. Fig. 1. shows the subcloning procedures and p-12BS was identified by agarose gel electrophoresis after digestion with restriction endonuclease (data not shown). The restriction endonuclease map of p-12BS is shown in Fig. 2.

Expression of the *cat-86* gene in p-12BS during growth of *B. subtilis* and *Bacillus* sp. YA-14

B. subtilis 207-25 harboring p-12BS was grown at 37°C in sporulation media in the presence of 0.1% glucose or 1% glucose. Periodically 3 m*l* samples were withdrown, sonicated and assayed for CAT and total protein. The appearance of internal spores in the cells grown in the presence of 0.1% glucose was estimated by phase-contrast microscopy.

CAT specific activity was revealed to be a maximum at 8 hours after the end of the exponential growth and the increase in the CAT began at approximately the same time that internal spores were first detected in the culture (Fig. 3). From this results, it is thought that the promoter of p-12BS is related to spore for-

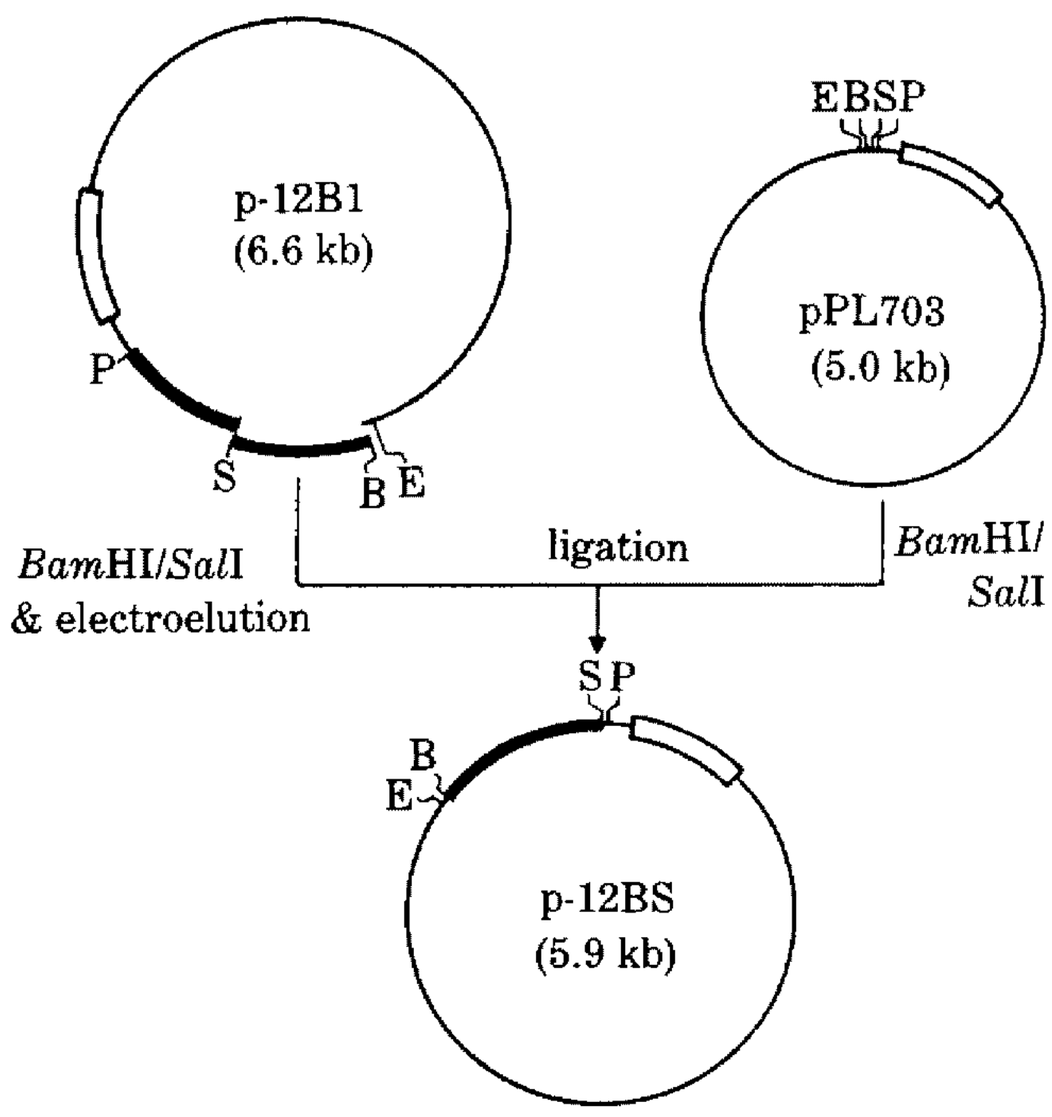

Fig. 1. Construction of plsmid p-12BS from p-12B1.
P; *Pst*I, S; *Sal*I, B; *Bam*HI, E; *Eco*RI, open box and closed box represent *cat-86* coding region and *Bacillus* sp. YA-14 DNA fragment, respectively, kb; kilobases.

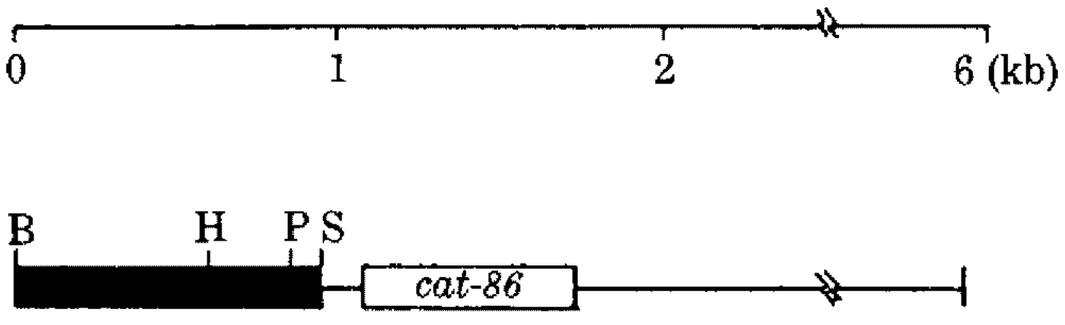

Fig. 2. Restriction map of plasmid p-12BS.
B; *Bam*HI, H; *Hind*III, P; *Pvu*II, S; *Sal*I

mation, of which expression stimulated at the onset of endospore formation.

In addition, CAT specific activity was considerably decreased in the medium with 1% glucose compared with in the medium with 0.1% glucose. It seems that this decrease is due to the existance of gene controlling catabolite repression in the promoter.

When alkali-tolerant *Bacillus* sp. YA-14 harboring p-12B1 was cultured, the similar results were obtained (Fig. 4).

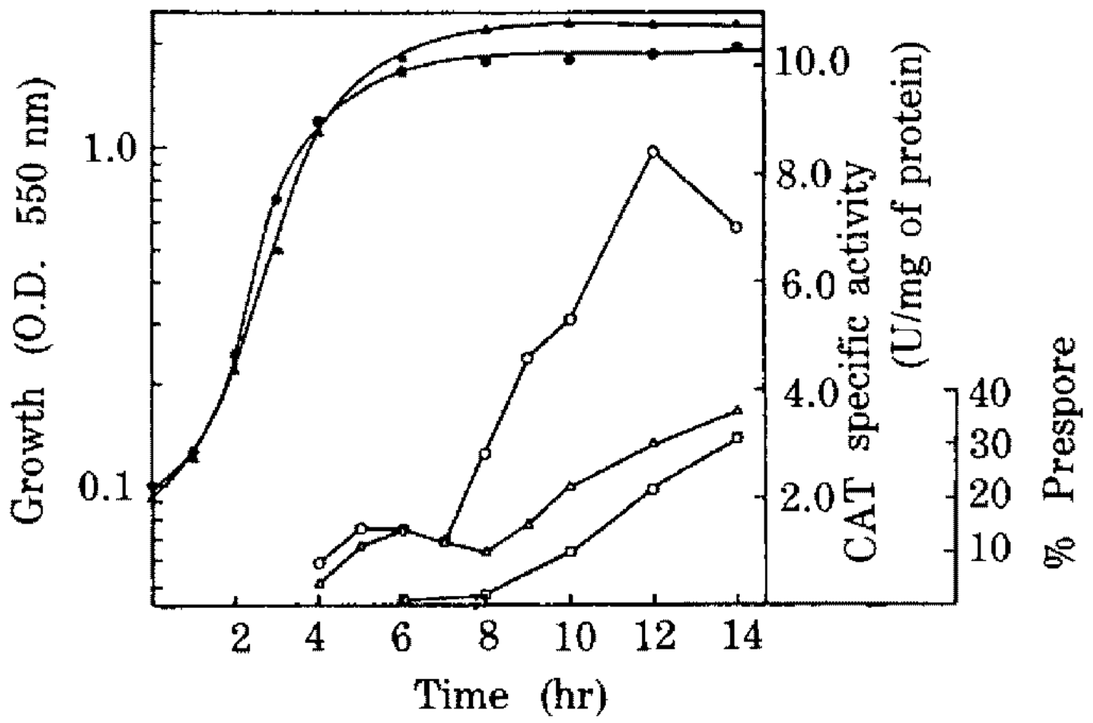

Fig. 3. CAT specific activity as a function of growth and endospore formation and effect of glucose concentration on CAT specific activity in *B. subtilis* 207-25 harboring p-12BS.
●, ▲; Cell growth in 2XSSG containing 0.1% glucose and 0.1% glucose, respectivety, ○, △; CAT specific activity in 2XSSG containing 0.1% glucose and 1.0% glucose, respectively, □; appearance of refractile prespore in 2XSSG containing 0.1% glucose

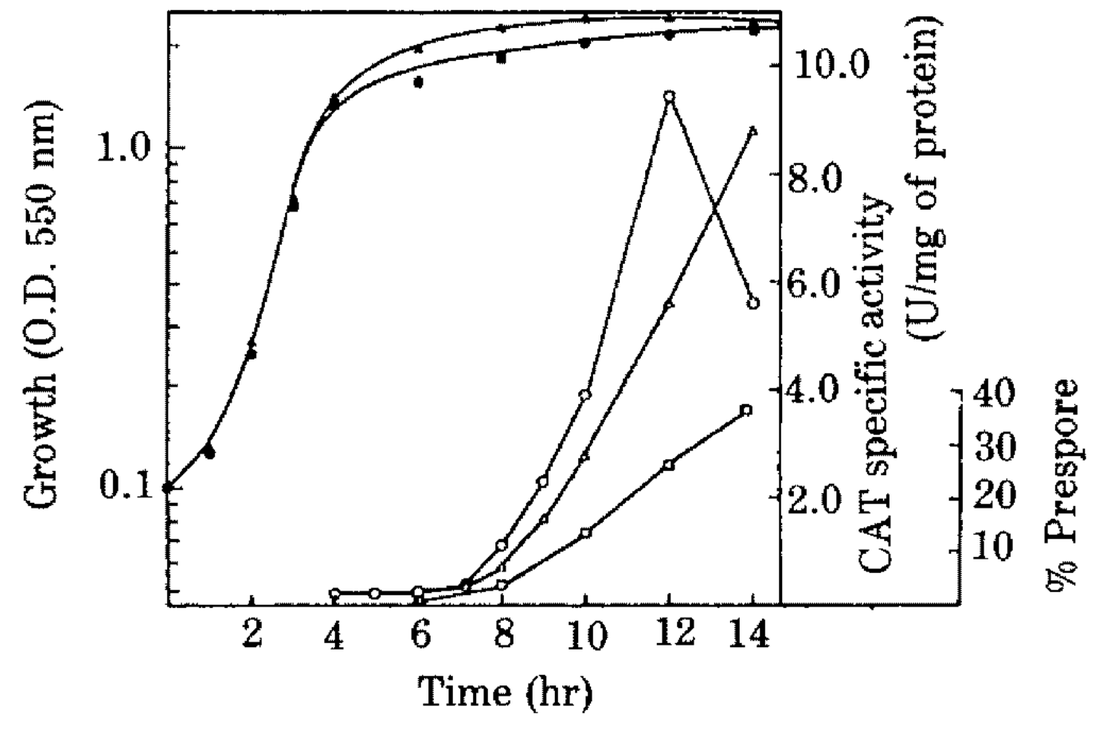

Fig. 4. CAT specific activity as a function of growth and endospore formation and effect of glucose concentration on CAT specific activity in *Bacillus* sp. YA-14 harboring p-12BS.
●, ▲; cell growth in 2XSSG containing 0.1% glusoce and 1.0% glucose, respectively, ○, △; CAT specific activily in 2XSSG containing 0.1% glucose and 1.0% glucose, respectively, □; appearance of refractile prespore in 2XSSG containing 0.1% glucose

Effect of *spoO* gene on the expression of CAT

More than 30 spore gene operons have been identified in *B. subtilis* by genetic mapping of Spo- mutation (19). The various sporulation mutations define genes whose functions are required for development. *spoO* mutations define those genes that function earliest in sporulation and an order of nine *spoO* genes has been suggested (19).

In order to determine the role of *spoO* gene in the expression of promoter, p-12BS was transformed into sporulation-positive strain of *B. subtilis* and 5 strains of its relevant mutant containing mutations in known

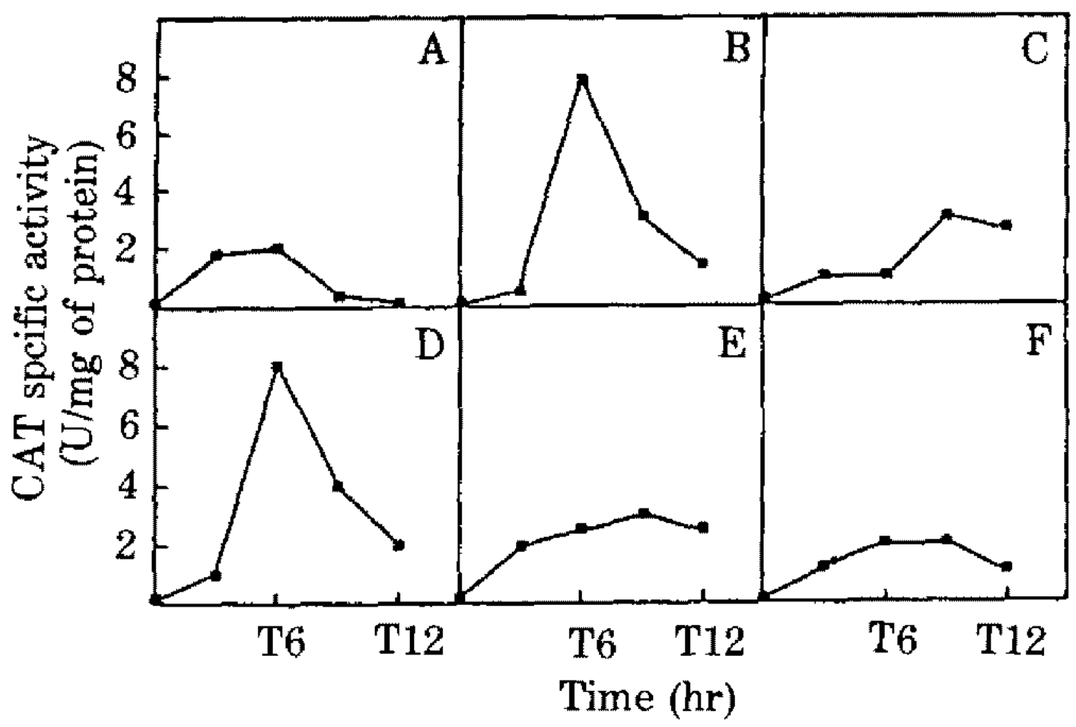

Fig. 5 Effect of various *spoO*mutation on CAT expression.
A; *B. subtilis* JH642 (*spo*$^+$), B; *B. subtilis* JH647 (*spoOE*), C; *B. subtilis* JH648 (*spoOB*), D; *B. subtilis* JH649 (*spoOF*), E; *B. subtilis* JH651 (*spoOH*), F; *B. subtilis* JH696 (*spoOJ*), harboring p-12BS, respectively.

spore genes and the expression of *cat-86* was tested. Plasmid containing strains were grown in sporulation media and periodically 3 m*l* samples were withdrawn, sonicated and assayed for CAT. Unexpectedly, the CAT specific activity of sporulation-positive strain of *B. subtilis* containing p-12BS was very low. This phenomenon seemed to be due to inter or intramolecular recombination of plasmid DNA, because p-12BS isolated from sporulation positive strain was somewhat large in melecular weight in comparison with p-12BS isolated from other *spoO* gene mutant or *B. subtilis* 207-25 (data not shown).

As shown in Fig. 5. The CAT specific activity of the *spoOE* strain (panel B) and the *spoOF* strain (panel D) were maximally expressed at 6 hours (T6) after the end of exponential growth and have a level of CAT activity similar to that of the *B. subtilis* 207-25. But the *spoOB, spoOH,* and *spoOJ* strains showed relatively the low activity of CAT. Thus the *spoOE* and the *spoOF* mutations have little or no effect on the expression of promoter, and the expression of promoter of the p-12BS was affected by the *spoOB, spoOH, spoOJ* gene product. In these results, we proposed that the promoter of p-12BS seemed to be related to spore formation.

요 약

알카리 내성 *Bacillus* sp. YA-14의 chromosomal DNA로부터 유래된 promoter를 subcloning하여 그 생화학적 특성과 포자형성과의 관계를 조사하였다. 알카리 내성 *Bacillus* sp.와 *B. subtilis*에서 promoter의 활성은 포자가 형성되기 시작하는 단계에서 증가하기 시작했다. 또한 배지내 glucose가 1.0%(w/v) 첨가시 promoter 활성이 억제되었다. 5가지의 *spoO* 유전자 중에서 3가지 유전자(*spoOB, spoOH, spoOJ*) 산물이 promoter 활성에 필요하였다.

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References

1. Doi, R.H.:*Arch. Biochem. Biophys.*,**214,** 772 (1982).
2. Losick, R. and J. Pero: *Cell,* **15,** 582 (1981).
3. Johnson, W.C., C.P. Jr. Moran and R. Losick: *Nature* (London), **302,** 800 (1983).
4. Williams, D.M., E.J. Duvall and P.S. Lovett: *J. Bacteriol.,* **146,** 1162 (1981).
5. Yu, J.H., B.T. Koo, I.S. Kong, Y.J. Chung and Y.S. Park: *Kor. J. Appl. Microbiol. Bioeng.,* **16,** 126 (1988).
6. Yu, J.H., B.T. Koo, Y.S. Park, Y.J. Chung, D.H. Bai and D.H. Oh: *Kor. J. Appl. Micorbiol. Bioeng.,* **16,** 343 (1988).
7. Yu, J.H., B.T. Koo and Y.J. Chung: *Kor. J. Appl. Micorbiol. Bioeng.,* **17,** 188 (1989).
8. Otozai, K. and K. Yamane: *Agric. Biol. Chem.,* **49,** 351 (1985).
9. Yu, J.H., Y.J. Chung, K.S. Chung and D.H. Oh: *Kor. J. Appl. Microbiol. Bioeng.,* **14,** 239 (1986).
10. Yu, J.H., Y.S. Park, J.M. Kim, I.S. Kong and Y.J. Chung: *Kor. J. Appl. Microbiol. Bioeng.,* **16,** 316 (1988).
11. Yu. J.H., D.C. Park, Y.J. Chung and I.S. Kong: *Kor. J. Appl. Microbiol. Bioeng.,* **17,** 154 (1989).
12. Chang, S. and S.N. Cohen: *Mol. Gen. Genet.,* **168,** 111 (1978).
13. Bott, K.F. and G.A. Wilson: *J. Bacteriol.,* **94,** 562 (1967).
14. Goldfarb, D.S., S.L. Wang, T. Kudo and R.H. Doi: *Mol. Gen. Genet.,* **191,** 319 (1983).
15. Shaw, W.V.: *Methods Enzymol.,* **43,** 737 (1975).
16. Lowry, O.E., N,J. Rosebrough, A.L. Farr and R.J. Randall: *J. Biol. Chem.,* **193,** 265 (1951).
17. Goldfarb, D.S., R.L. Rodriquez and R.H. Doi: *Proc. Natl. Acad. Sci. USA,* **79,** 5886 (1982).
18. Maniatis, T., E.F. Fritsch and J. Sambrook: "*Molecular cloning, A laboratory manual*", Cold Spring Harbor Laboratory (1982).
19. Piggot, P.J. and J.G. Coote: *Bacteriol. Rev.,* **40,** 908 (1976).

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