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Extracellular Production of Alpha-Interferon by Recombinant *Escherichia coli*: Part Ⅱ. The Growth Behavior of the Recombinant Cells

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유전자 재조합 대장균을 사용한 Alpha-Interferon의 생산과 분비: 제2부. 재조합 균주의 생장특성

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ABSTRACT

The growth behavior of recombinant *Escherichia coli* cells having plasmid pIF-Ⅲ-B, which carries human alpha-interferon gene under the control of *lpp* promoter, *lac* promoter and *lac* operator, was studied by using of various *E. coli* host strains. Expression of the alpha-IFN gene is controllable by using inducer IPTG because the plasmid also contains *lacI* gene which produces *lac* repressors. The repressors block the transcription of alpha-IFN gene.

There were considerable differences in cell growth according to the host strains used. Cell growth was inhibited not only by plasmid pIF-III-B itself but also by the induction of alpha-IFN gene expression. Growth inhibition caused by the plasmid itself was more serious than that caused by the induction of alpha-IFN gene expression.

INTRODUCTION

Recombinant DAN technology permits large-scale production of eukaryotic proteins in bacterial host strains. Even though cloning a foreign gene in a particular host systems is major task, cultivation of the recombinant cells harboring hybrid plasmids for overproduction of cloned gene products is also as important as cloning itself.

There seems to be some relationship between characteristics of recombinant plasmids and cell growth behavior, that cells with plasmid grow more slowly than cells without plasmids, and that in case of cultures containing highly amplified plasmid, active expression of cloned foreign gene leads host cells even to death.

We have previously reported the construction of vectors for alpha-IFN gene expression in part Ⅰ(1). By using one of these plasmid vectors, pIF-Ⅲ-B which is shown in Fig. 1, we studied the effect of the plasmid in various host strains and the induction of alpha-IFN gene expression on cell growth behavior.

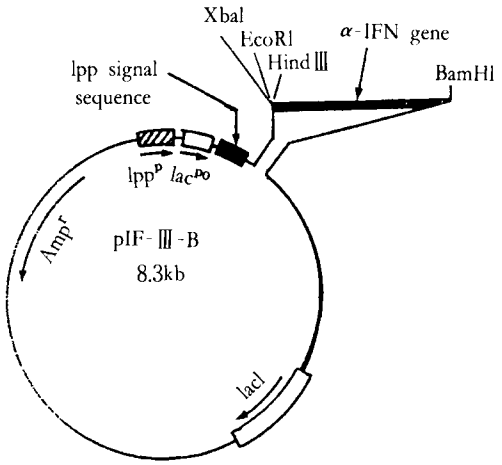

Fig. 1. Structure of final alpha-IFN expression vector pIF-III-B.

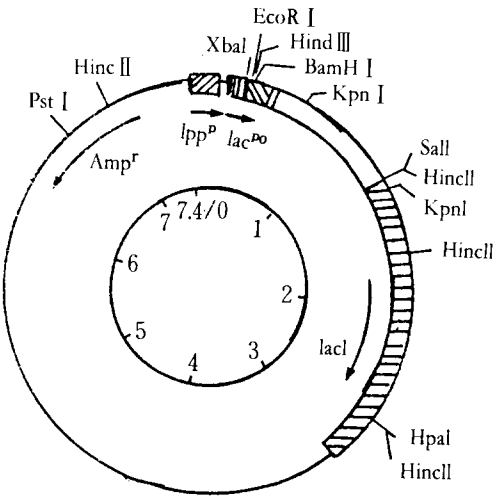

Fig. 2. Structure of plasmid pIN-III B3.

MATERIALS AND METHODS

Bacterial strains and plasmid and culture conditions

E. coli strains used throughout this study are listed in Table 1. The bacteria were used as host strains for plasmid pIF-III-B carrying a alpha-IFN gene. Plasmid pIF-III-B was constructed by inserting alpha-IFN gene into plasmid pIN-IIIB3(2) which was provided by Masayori Inouye (State Univ. of NY at Stony Brook). The map of this plasmid is shown in Fig. 2.

All the *E. coli* strains were cultivated in LB medium at 37℃. Rotary flask shaker(New Brunswick Sci.) set at 180 rpm was used to investigate growth behaviors of cells harboring the plasmid pIF-III-B. Cell growth was checked by measuring optical density with spectrophotometer at wavelength of 600 nm.

Induction of gene expression

For the induction of alpha-IFN gene expression in *E. coli* host strains, 1mM of IPTG was added to the media. IPTG was dissolved in distilled water to make 0.1M final concentration and sterilized with membrane filtration. The stock solution was kept at −20℃ in aliquots.

Table 1. *E. coli* strains and genotypes

Strain	Genetic markers	Reference
C600	F^-, thi−1, thr−1, leuB6 lacY1, tonA21, supE44, λ^-	3
HB101	F^-, hsd20(r_B^-, m_B^-), recA13, ara-14, proA−2, lacY−1, galK−2, rpsL20(Sm^r), xyl	4
JE5505	F^-, lpp−2, pps, his, proA, argE, thi, gal, lac, xyl, mtl, tsx	5
RB791	W3110 $lacI^qL8$, endA1, gyrA96, thi, hsdR17,	6

Transformations

Transformation of *E. coli* strains with plasmid DNAs was performed as described by D.A. Morrison(7). Transformants were screened on LB plate supplemented with 50 μg / ml of ampicillin.

Preparation of recombinant cell extracts for the assay of alpha-IFN

Cell extract preparation was based on the method of Nagata(8). The harvested cells from 50ml of cultur were washed with 5ml of washing buffer [50mM Tris (pH 8.0), 30mM NaCl] twice or three times and resuspended in 5ml of lysozyme solution [75 mM EDTA, 75 mM Tris (pH8.0), 0.3 M Sucrose, 3mg / ml of lysozyme]. After 30 min of incubation in ice bath the cells were frozen (−70℃) and thawed (40℃) repeatedly five or six times until complete lysis of cells was accomplished. After centrifugation at 200,000 g force for 1 hr, the supernatant was taken as extract sample.

Determination of alpha-IFN activity

Various methods for alpha-IFN assay were described in detail by Pestka, S.(9). We followed the method described by Rubinstein, S.(10). This assay is based on the measurement of a parameter associated with cytopathic effect on host animal cells.

RESULTS

Growth behavior of various *E. coli* strains harboring plasmids

Various *E. coli* strains were transformed with plasmid pIF-III-B, to study the effect of alpha-IFN production on their growth and to find most suitable host strains for the production of alpha-IFN. Growth profiles of each strains were shown in Fig. 3 and Fig. 4. Also indicated in Table 2, specific growth rate and final OD value were different according to host strains used. We could find no proportional relationship between maximum specific cell growth rate and maximum cell density.

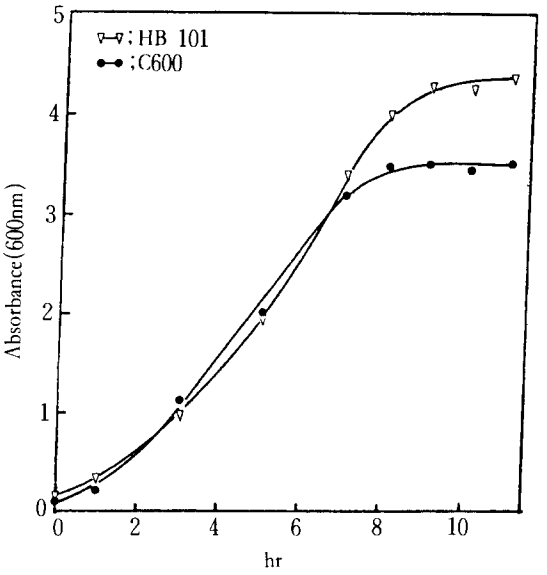

Fig. 3. Growth curve of recombinant *E. coli* harboring plasmid pIF-III-B in LB medium supplemented with 1 mM IPTG.

Cloned alpha-IFN gene product formation

As an attempt to select the most appropriate host strain for the production of alpha-IFN, we determined alpha-IFN activities from the culture of each recombinant *E. coli*

Table 2. Growth of various *E. coli* strains harboring plasmid pIF-III-B in the presence of IPTG and ampicillin

Host	μ_{max} (hr^{-1})	OD_{max} (600nm)	Maximum cell density (g / l)
C600	0.99	3.5	1.44
HB101	0.68	4.4	1.74
JE5505	1.01	3.3	1.44
RB791	0.93	4.3	2.03

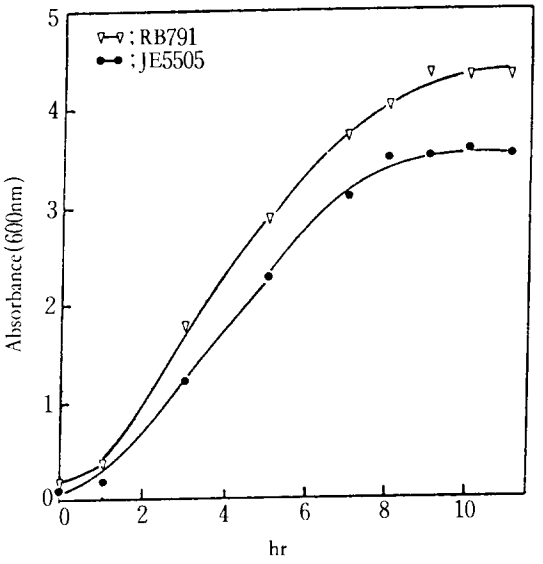

Fig. 4. Growth curve of recombinant *E. coli* harboring plasmid pIF-Ⅲ-B in LB medium supplemented with 1 mM IPTG.

strains. Alpha-IFN activities based on unit volume of cell extract, unit volume of the culture and unit mass of dry cell were illustrated in Table 3. As it can be seen in Table 3, all the strains except C600 showed similar activities of 1.6×10^4 IU per ml of culture. Meanwhile, JE5505 showed highest activity based on unit cell mass. It seems to be related with the genotype of *lpp*-2, in other words, JE5 505 is unable to produce lipoprotein while alpha-IFN gene is under the control of *lpp* promoter.

Effect of plasmid pIF-Ⅲ-B and induction of gene expression on cell growth

E. coli JE5505 was used as a host strain in order to investigate factors that affect the cell growth. As illustrated in Fig. 5, there was little difference in growth rate of the host strain harboring plasmid pIF-III-B regardless of whether an inducer, IPTG was added to the medium. As it can be seen in Fig. 6, considerable difference in the cell growth was observed between the cells with plasmid and the cells without plasmid. These results were tabulated in Table 4. From these observations, it was concluded that the plasmid pIF-III-B could act as a serious stress to the host strain, while once the host strain harbors the recombinant plasmid, the recombinant cell feels nearly the same extent of stress irrespective of the induction of gene expression.

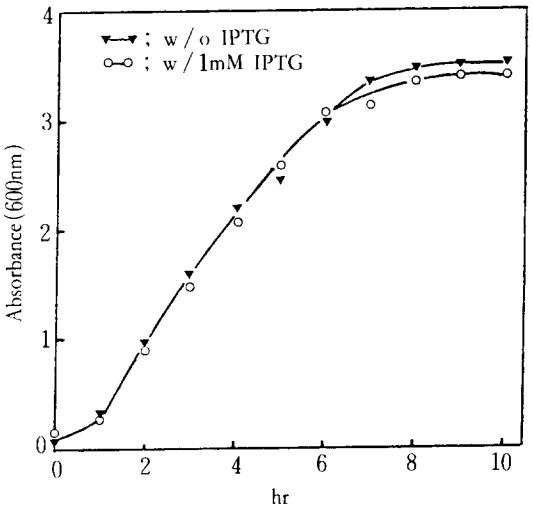

Fig. 5. Growth curve of recombinant *E. coli* JE5 505 harboring plasmid pIF-Ⅲ-B. (w/o: without, w/ : with)

Table 3. Total activity of alpha-IFN from the cultures of various *E. coli* strains harboring recombinant plasmids pIF-Ⅲ-B

Strain	IU / ml of Extract	IU / ml of culture	IU / g of dry cell
C600	0.8×10^5	0.8×10^4	5.6×10^6
HB101	1.6×10^5	1.6×10^4	9.2×10^6
JE5505	1.6×10^5	1.6×10^4	11.1×10^6
RB791	1.6×10^5	1.6×10^4	7.9×10^6

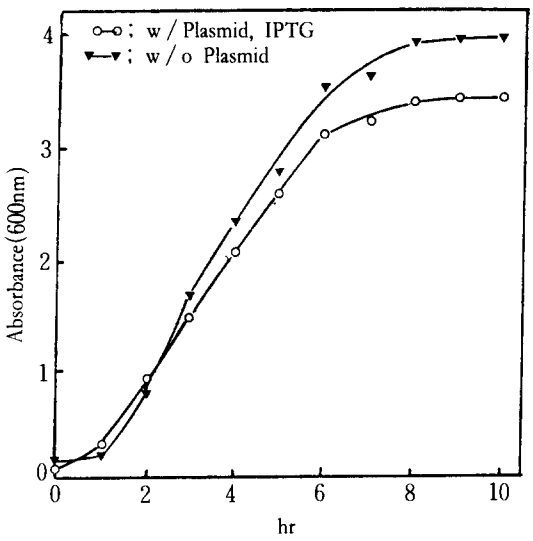

Fig. 6. Growth curve of *E. coli* JE5505 with and without plasmid pIF-Ⅲ-B. (w / ;with, w / o; without)

We investigated the plasmid stability of the culture by plating the culture on LB agar and transferring the single colonies grown on LB agar to ampicillin-added LB agar. The plasmid stability of the culture was found to be nearly 100% during the culture time. Because the plasmids were stably maintained during the culture period, the difference in the cell growth may be ascribed to reduction in host cell biosynthetic activity for native components as a result of redirection, by the plasmid, of the host cell biosynthetic potential mainly toward the replication of the plasmid and partly toward plasmid-encoded gene product formation.

DISCUSSION

When various *E. coli* strains were used as hosts for the plasmid pIF-III-B which carries alpha-IFN gene, each strain showed different growth rate. JE5505 which can not product lipoprotein produced the highest titer of alpha-IFN per unit cell mass.

While there was considerable difference in host cell growth between blank cells without plasmid and cells carrying plasmid even though an inducer was not added to the culture media, there could be found nearly no additional growth inhibitory effect of the induction of alpha-IFN expression by adding IPTG. It explains that replication of the recombinant plasmid acts as a kind of stress on host cell and if the host strain once carries the recombinant plasmid, expression of alpha-IFN gene almost dose not acts as an extra stress on the host cells. This seems to be possibly related with the secretion of much of alpha-IFN produced.

ABBREVIATIONS

Amp^r: ampicillin resistant, IFN: interferon, IPTG: isopropyl-β-D-thiogalactopyranoside, IU: international unit, kb: kilo base pairs, *lac*: lactose, LB: Luria Bertani, *lpp*: lipoprotein, OD: optical density, Tc^r: tetracycline resistant, X-gal: 5-bromo-4-chloro-3-indolyl-β-D-galactoside

요 약

대장균의 lipoprotein promoter, lactose promoter 및 operator 와 lipoprotein의 signal sequence를 가지는 vector 에 alpha-IFN 유전자가 cloning된 plasmid pIF-Ⅲ-B를 여러종류의 대장균 숙주 세포에 형질전환하여 alpha-IFN

Table 4. Effects of plasmid pIF-Ⅲ-B and inducer IPTG on the the growth of host strain JE5505

Culture condition	$\mu_{max}(hr^{-1})$	OD_{max}(600nm)	Maximum cell density(g / l)
blank cell*	1.34	3.9	1.70
plasmid, Amp	1.14	3.5	1.52
plasmid, Amp, IPTG	1.01	3.4	1.48

*: JE5505 not harboring plasmid

의 생산성, 생장특성을 조사하였다. 또한 plasmid 자체와 cloning된 alpha-IFN 유전자의 발현유도가 세포생장에 미치는 영향을 조사하였다.

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