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Effect of Induction Temperature on the P_L Promoter Controlled Production of Recombinant Human Interleukin-2 in *Escherichia coli*

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The effect of induction temperature on fermentation parameters has been investigated extensively using *Escherichia coli* M5248[pNKM21], a producer of recombinant human interleukin-2 (rhIL-2). In this recombinant microorganism, the gene expression of rhIL-2 is regulated by the cI857 repressor and P_L promoter system. The recombinant fermentation parameters studied in this work include the cell growth, protein synthesis, cell viability, plasmid stability, β-lactamase activity, and rhIL-2 productivity. Interrelationships of such fermentation parameters have been analyzed through a quantitative assessment of the experimental data set obtained at eight different culture conditions. While the expression of rhIL-2 gene was repressed at culture temperatures below 34℃ with little effect on other fermentation parameters, under the conditions of rhIL-2 production (36~44℃) the cell growth, plasmid stability, and β-lactamase activity were, as induction temperature was increased, more profoundly reduced. Although the rhIL-2 content in the insoluble protein fraction was maximum at 40℃, total rhIL-2 production in the culture volume was found to be highest at the induction temperature of 36℃. This was in contrast to the previously known optimum induction temperature of the P_L promoter system (40~42℃). Explanations for such a discrepancy have been proposed based on a product formation kinetics, and their implications have been discussed in detail.

While many reports on the cloning and expression of recombinant DNA have been published, studies on the fermentation process of recombinant cells are relatively limited. Since recombinant fermentation parameters such as cell growth, protein formation, recombinant cell viability, plasmid stability, and expression of recombinant genes are highly interrelated, these parameters must be carefully studied and evaluated when the recombinant fermentation process is to be optimized (18).

We have previously reported the preliminary results on fermentation conditions of recombinant *E. coli* M5248[pNKM21], including design of the medium composition, effect of induction time, and computer controlled temperature induction (2, 7). *E. coli* M5248[pNKM21] produces the mutein of recombiant human interleukin-2(rhIL-2) in which the cysteine residue at amino acid 125 of native rhIL-2 has been replaced with serine (4, 5). In this recombinant microorganism the cI857 repressor gene is carried on *E. coli* chromosome and the gene expression of rhIL-2 is under control of the P_L promoter of bacteriophage lambda. It has been well known that a temperature-sensitive cI857 repressor is active at low temperatures (28~30℃) and represses transcription from the P_L promoter while at high temperatures (40~42℃) the cI857 repressor is inactive and transcription from the promoter is derepressed (11, 15, 23).

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The P_L promoter of bacteriophage lambda have been used extensively for the expression of the foreign genes in *E. coli* (1, 3, 12, 16, 17, 20-22). However, there are few reports on the detailed examination of temperature effect on recombinant fermentation parameters in this system. Siegel and Ryu (20) have investigated the effect of temperature on the production of P_L-promoted gene product. As temperature was increased from 38.2℃ to 39.2℃, a 40-fold increase in *trp*A productivity was observed. Similarly, Sugimoto *et al.* (22) reported that the production of β-galactosidase increased with the increase of induction temperature in the range of 36℃ to 42℃, and was maximum at 42℃.

For the production of target product using a recombinant microorganism, however, optimal fermentation conditions might not be identical and will be dependent on the nature of the cloned-gene product, properties of cloning vectors and genetic background of the host cell. In other words, the influence of culture temperature on the fermentation parameters would be different even the case where the same cI857 repressor and P_L promoter system is employed for the regulation of cloned-gene expression.

In view of this background, we have investigated the effects of culture temperature on recombinant fermentation parameters of cell growth, protein synthesis, recombinant cell viability, plasmid stability, β-lactamase activity, and rhIL-2 productivity using an rhIL-2 producing recombinant strain, *E. coli* M5248[pNKM21]. Furthermore, we have analyzed the interrelationships of the aforementioned fermentation parameters by quantitating the experimental data. The results shown in this paper may provide a more comprehensive understanding on the interaction between the cloned-gene expression and the host-cell metabolic activity.

MATERIALS AND METHODS

Recombinant Microorganisms

The host strain used was *Escherichia coli* M5248 (*bio* 275 cI857 ΔHI), which contains the cI857 gene on its chromosome. Recombinant *E. coli* M5248[pNKM21] (*E. coli* M5248 harboring the plasmid pNKM21) was used as an rhIL-2 producing strain. Plasmid pNKM21 was constructed by inserting the coding sequence of human interleukin-2 into the *Bam*HI site of pAS1 vector as described elsewhere in detail (4, 5). Expression of the rhIL-2 gene is under control of the P_L promoter of bacteriophage lambda.

Cuture Medium and Fermentation Condition

Luria(LB) medium was used for seed culture of recombinant cells. F medium which was used as a main fermentation medium contains per liter (7): 50 g glucose, 10 g NH_4Cl, 1.2 g sodium citrate, 1.0 g $MgCl_2 \cdot 6H_2O$, 3.0 g K_2HPO_4, 0.4 g K_2SO_4, 15 mg $FeCl_3 \cdot 6H_2O$, 1.0 g yeast extract, and 10 m*l* of trace element solution (3 μM MoO_3, 400 μM H_3BO_3, 10 μM $CuSO_4$, 10 μM $ZnSO_4$, and 80 mM $MnCl_2$). Glucose and other medium components were sterilized seperately at 121℃ for 15 min and the initial pH of media was adjusted to 7.0 with 4 N NaOH.

Jar fermentation of recombinant cells was carried out in a 5-liter fermentor (Korea Fermentor Co.) equipped with a DO analyzer and a pH controller. One hundred m*l* of seed culture cultivated at 30℃ for 16 h in shake flasks was transferred into a fermentor containing 1.9 liter of F medium. Air flow rate and agitation speed were 1.5 vvm and 400 rpm, respectively. The pH was controlled automatically at 7.0 with 4 N NaOH. For rhIL-2 inductioin, the culture temperature was shifted from 30℃ to a predetermined temperature after 8 h cultivation at 30℃. Fermentation in a jar fermentor was carried out in a batch mode operation.

Fractionation of Intracellular Protein

For the determination of rhIL-2 content rhIL-2 was partially purified from culture broth by the following procedure since rhIL-2 accumulates as inclusion bodies in the *E. coli* cytoplasm (2). Cells (20~40 mg) harvested from culture broth were resuspended with 2.5 m*l* of 50 mM Tris-HCl (pH 8.3) and treated with lysozyme (200 μg/m*l*) for 30 min at 25℃. The cells in the suspension were disrupted with a sonicator (Model VC 250, Sonics and Materials Inc., USA) while keeping the suspension on ice to prevent heating-up. The lysate was used for the determination of total intracellular protein. In order to fractionate the intracellular proteins further, 1 m*l* of lysate was centrifuged at 5,000 g for 10 min. The pellet harvested was washed with 1 m*l* of Tris-HCl buffer and centrifuged again at the same condition. Finally the pellet resuspended with Tris-HCl buffer was used for the determination of rhIL-2 content in the insoluble residues, while the supernatant solution collected from the preceding steps was used for the assay of β-lactamase activity. The protein concentration of the insoluble fraction and the supernatant fraction was determined separately, and the protein in each fraction was referred as insoluble protein and soluble protein, respectively.

Analytical Methods

Glucose concentration was determined by the DNS method (14). Cell growth was monitored by measuring the optical density of culture broth at 540 nm with a spectrophotometer (Spectronic 20, Baush & Lomb). The cell concentration was also determined by measuring the dry cell weight. The ratio of dry cell weight (g/*l*) to optical density was 0.47. The viability of recombinant cells was determined by counting the colony number

on LB agar plates. Protein assay was followed by the Lowry method (13). The activity of β-lactamase during cultivation of recombinant cells was measured by iodine-titration method (19). Hydrolysis of 1 μmol of ampicillin per minute was defined as one unit of enzyme activity.

Stability of recombinant cells, expressed as the ratio of plasmid-harboring cells to total recombinant cells, was determined by the replica-plating method. Colony isolates grown in LB agar plates were tooth-picked into LB agar plates containing 100 μg/ml of ampicillin. After incubating for two days at 30℃, the fraction of plasmid-harboring cells was determined by counting the number of ampicillin-resistant cells.

The content of rhIL-2 was determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the Laemmli procedure (6). SDS-PAGE was carried out using 14% polyacrylamide gel. Protein band corresponding to rhIL-2 (M.W. 15.0 kd) was identified from SDS-PAGE of purified rhIL-2 and marker proteins of known size. The fraction of rhIL-2 in the insoluble protein residues was determined by scanning SDS-PAGE gels using a densitometer (Sebia, France).

RESULTS AND DISCUSION

Experimental Design and Summary of Data

In this work, the effect of induction temperature on fermentation parameters has been extensively investigated using a recombinant *E. coli* M5248[pNKM21]. Conventionally, the expression of cloned-gene product which is regulated by the cI857 repressor and P_L promoter system has been induced by shifting the culture temperature from 28~32℃ to 40~42℃ (9-12, 16). In the present study the induction temperature was subdivided into six temperatures ranging from 34℃ to 44℃ with an interval of 2℃. In this temperature-shift(TS) mode of operation the culture temperature was changed from 30℃ to a predetermined induction temperature after 8 hr cultivation at 30℃. For comparison, recombinant cells were also cultivated under the constant-temperature(CT) mode of operation, i.e., cells were grown at either 30℃ (repressed condition) or 42℃(induction condition) throughout the culture time without a temperature shift. As a consequence, the experiments were carried out at a total of eight different culture conditions. For each run of fermentation, the following parameters have been determined: (i) cell concentration, (ii) total protein concentration, (iii) insoluble protein concentration, (iv) soluble protein concentration, (v) the number of viable cells, (vi) the fraction of plasmid-harboring cells, (vii) β-lactamase activity, and (viii) rhIL-2 content.

All the experimental data obtained in this work are summarized in Fig. 1. In this figure, a total of 64 sets of experimental results are shown (total protein and insoluble protein concentrations are plotted together). Culture temperatures corresponding to (a)~(h) in Fig. 1 are as follow: (a) 30℃ (CT mode), (b) 34℃ (TS mode), (c) 36℃ (TS mode), (d) 38℃ (TS mode), (e) 40℃ (TS mode), (f) 42℃ (TS mode), (g) 44℃ (TS mode), and (h) 42℃ (CT mode). In the followings the effect of culture temperature on recombinant fermentation patterns and the interrelationships of fermentation parameters have been analyzed in detail.

Cell Growth and Protein Synthesis

First, the effect of induction temperature on the cell growth and protein synthesis of the recombinant *E. coli* M5248[pNKM21] have been analyzed. While the cell growth was very excellent at 30℃ (repressed condition), the cell growth at 42℃ (induction condition) was severely inhibited ((a) and (h) in the first panel of Fig. 1). When the culture temperature was shifted from 30℃ to a different induction temperature (from 34℃ to 44℃) after 8 h cultivation at 30℃, both the cell growth rate and the final cell concentration were reduced as temperature was increased. On the other hand, when the host strain (*E. coli* M5248) was cultivated at either 30℃ or 42℃ under constant temperature mode, or even under temperature shifting mode, no significant difference in cell growth was found as described in an accompanying paper (8). This result implies that the expression of rhIL-2 gene reduced the growth of recombinant cells due to the stress on the metabolism of host organism.

Since rhIL-2 is produced as intracellular protein in the recombinant cells, total protein synthesis has also been examined. As shown in Fig. 1, the production pattern of total intracellular protein was in parallel with that of cell growth. Total protein concentration was proportional to the cell concentration with a correlation coefficient (γ) of 0.986, and total protein content of recombinant cells was 56± 5% of dry cell weight on average irrespective of culture temperature (Fig. 2).

Insoluble Protein Formation

From the experiments on SDS-PAGE analysis of rhIL-2, it was found that rhIL-2 could be detected only in the insoluble residues of cell lysates (Fig. 3). In order to examine the patterns of the insoluble protein formation under different culture conditions, we further fractionated the lysate and determined the protein concentration in the insoluble fraction and in the soluble fraction separately. The results are shown in the second and the third panels of Fig. 1.

Preliminary experiments indicate that the sonication procedure affect the result, and therefore the effects of the size of a probe and the number of sonication frequencies on fractionation of insoluble protein have been carefully investigated. The results are summarized in Ta-

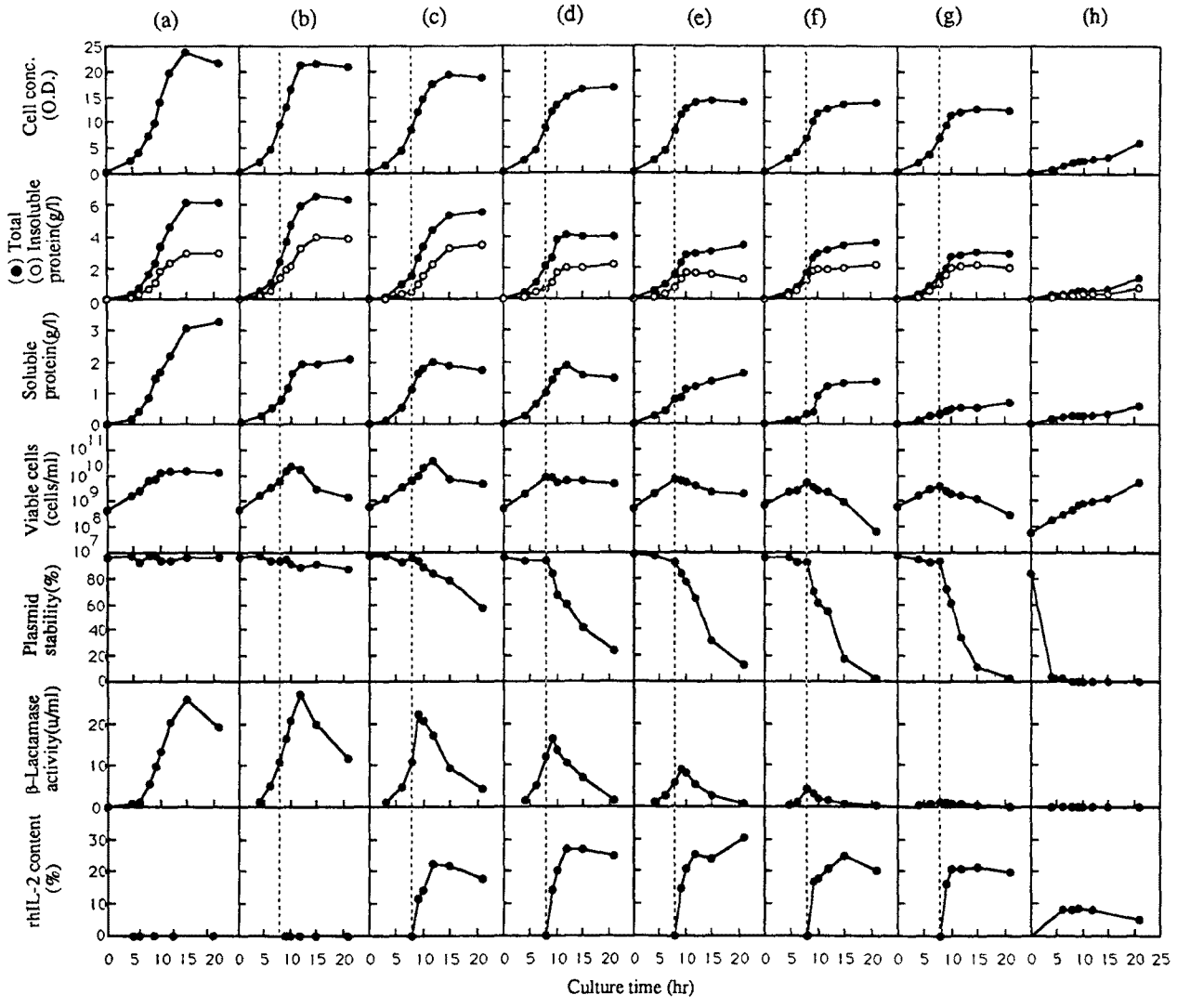

Fig. 1. Effect of culture temperature on fermentation parameters of *E. coli* M5248[pNKM21].
Culture conditions are: (a) 30℃ (CT mode), (b) 34℃ (TS mode), (c) 36℃ (TS mode), (d) 38℃ (TS mode), (e) 40℃ (TS mode), (f) 42℃ (TS mode), (g) 44℃ (TS mode), (h) 42℃ (CT mode). Details are described in the text.

ble 1. The protein concentration in the insoluble fraction, as the frequency of the sonication step was increased, was decreased with a concomitant increase of the rhIL-2 content. Analysis of the insoluble residues on SDS-PAGE, however, showed that total amount of rhIL-2 in cell lysates was not affected by the differences in the sonication procedure. On the other hand, the fraction of insoluble residues was lower with a large probe. In view of these results, standardization of the sonication procedure would be required for proper reproducibility. In all subsequent experiments, therefore, the cell suspension was sonicated five times with 1 min intervals using a microprobe.

The relationship between total protein synthesis and insoluble protein formation is depicted in Fig. 4(a). For a comparison of experimental accuracy, the sum of insoluble and soluble protein is also plotted against total protein (Fig. 4(b)). Each fraction has been determined separately. Although the data shown in Fig. 4(a) are somewhat scattered, it appears that the amount of insoluble protein becomes to increase at higher temperatures. For example, the average values of insoluble protein fraction to total protein were 65.3% and 70.0% when the culture temperature was shifted to 42℃ and 44℃, respectively. These clearly contrast to the average value of 55.4% obtained from the regression of all data points. Such phenomena might be due to the production of rhIL-2 as inclusion bodies, although further experiments on this

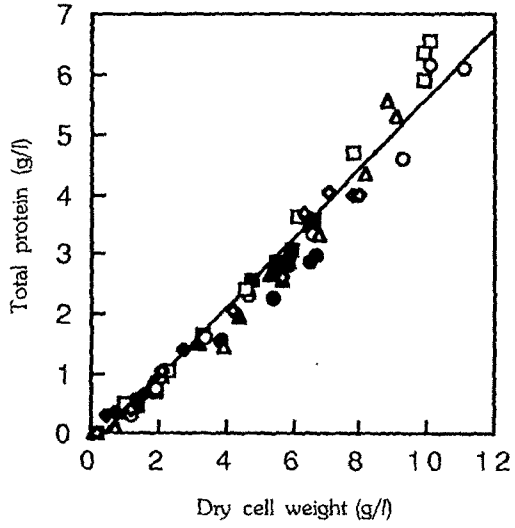

Fig. 2. Relationship between total intracellular protein and dry cell wieght.
Culture conditions are: (○) 30℃ (CT mode), (□) 34℃ (TS mode), (△) 36℃ (TS mode), (◇) 38℃ (TS mode), (●) 40℃ (TS mode), (■) 42℃ (TS mode), (▲) 44℃ (TS mode), (◆) 42℃ (CT mode).

aspect should be carried out.

Cell Viability and Plasmid Stability

Number of viable recombinant cells, more specifically the number of plasmid-harboring viable cells, is one of the critical factors for production of a desired cloned-gene product using recombinant cells. The changes in cell viability and the fraction of plasmid-harboring cells during cultivation of *E. coli* M5248[pNKM21] under different culture temperatures are shown in the fourth and fifth panels of Fig. 1, respectively.

The viability of cells as well as the plasmid stability was changed with culture temperature. At the induction temperature below 36℃ the number of viable cells increased further during 3~4 h after a temperature shift, whereas the viability of the recombinant cells above 38℃ was decreased upon induction of rhIL-2 production. When the cells were grown under constant temperature mode, the population of total viable cells at 30℃ was mostly consisted of plasmid-harboring cells while at 42℃ plasmid-harboring cells were rapidly lost and therefore total viable cells were composed mainly of plasmid-free cells. The recombinant cells were stably maintained during cultivation at temperatures lower than 34℃. Under the conditions that the expression of rhIL-2 gene was induced (between 36℃ and 44℃) plasmids were lost more rapidly as the induction temperature was increased.

Since the formation of cloned-gene products occurs only in plasmid-harboring cell populations, the number of plasmid-harboring cells in culture volume has been

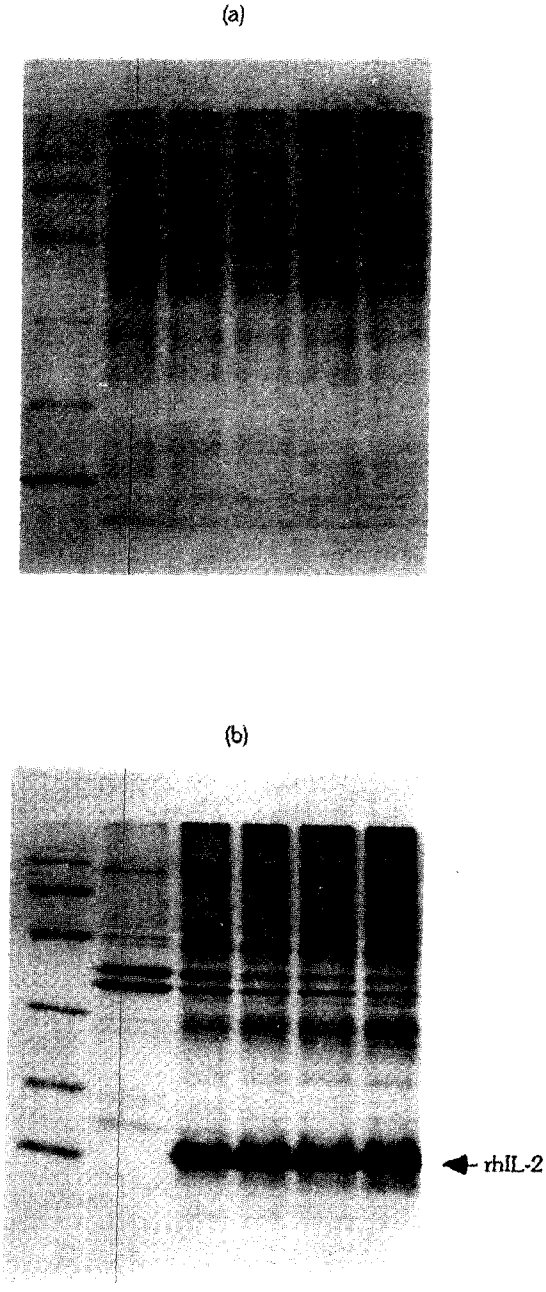

Fig. 3. SDS-PAGE of the fractionated cell lysate: (a) soluble protein fraction, (b) insoluble protein fraction.
Fractionation of total intracellular protein was carried out as described in the text. *E. coli* M5248[pNKM21] was cultivated under temperature-shift mode and the culture temperature was changed from 30℃ to 42℃ after 8 hr cultivation at 30℃. Each lane from the left to the right represents marker protein, sample for repressed condition (30℃), and samples for derepressed condition (42℃) at the culture time of 9, 10, 12, 15 h, respectively. The molecular weights of protein standard size markers are: 92.5, 66.2, 45.0, 31.0, 21.5, and 14.4 kD from the top.

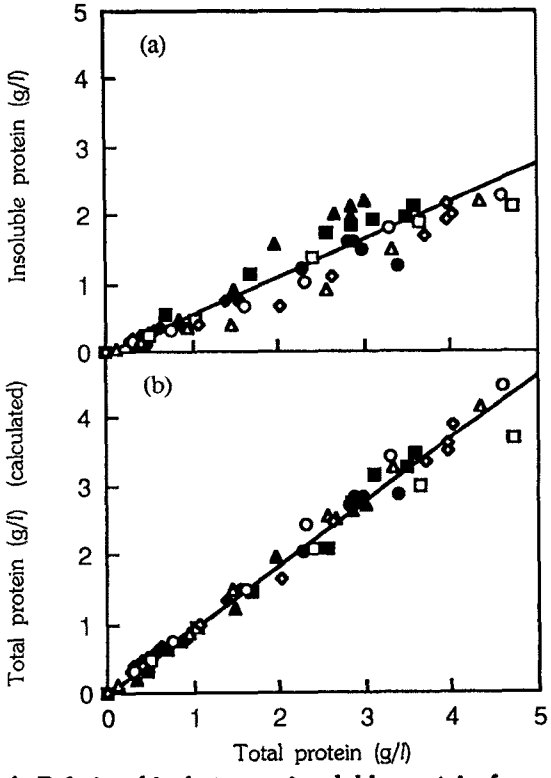

Fig. 4. Relationship between insoluble protein formation and total protein synthesis. (a) insoluble protein concentration vs. total protein concentration, (b) calculated total protein concentration vs. experimentally determined protein concentration.
Culture conditions are: (○) 30℃ (CT mode), (□) 34℃ (TS mode), (△) 36℃ (TS mode), (◇) 38℃ (TS mode), (●) 40℃ (TS mode), (■) 42℃ (TS mode), (▲) 44℃ (TS mode), (◆) 42℃ (CT mode).

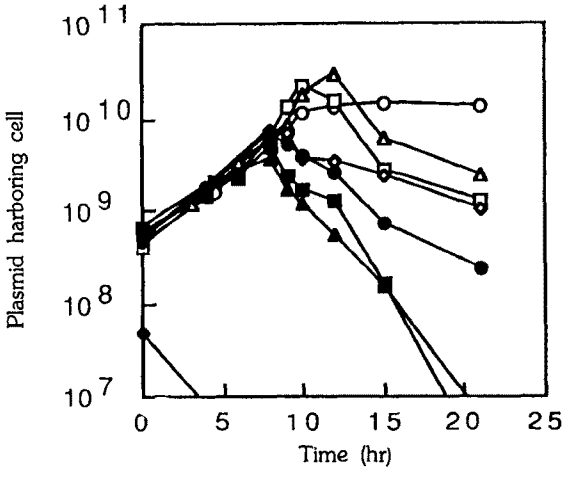

Fig. 5. Effect of culture temperature on total number of plasmid-harboring cells in the culture.
Culture conditions are: (○) 30℃ (CT mode), (□) 34℃ (TS mode), (△) 36℃ (TS mode), (◇) 38℃ (TS mode), (●) 40℃ (TS mode), (■) 42℃ (TS mode), (▲) 44℃ (TS mode), (◆) 42℃ (CT mode).

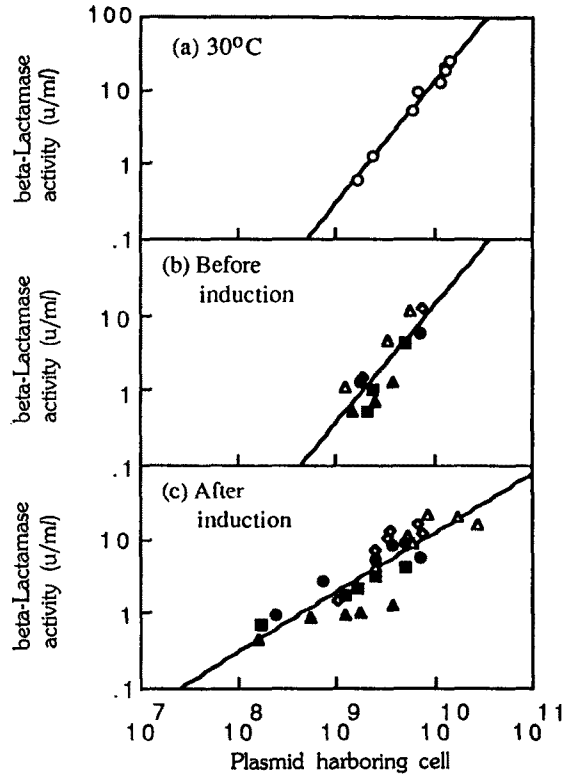

Fig. 6. Relationship between β-lactamase activity and the number of plasmid-harboring cell.
Culture conditions are: (a) CT mode (30℃), (b) and (c) TS mode (36~44℃). Data obtained under TS mode are divided into two groups: (b) before induction, (c) after induction. Legends are: (○) 30℃ (CT mode), (△) 36℃ (TS mode), (◇) 38℃ (TS mode), (●) 40℃ (TS mode), (■) 42℃ (TS mode), (▲) 44℃ (TS mode).

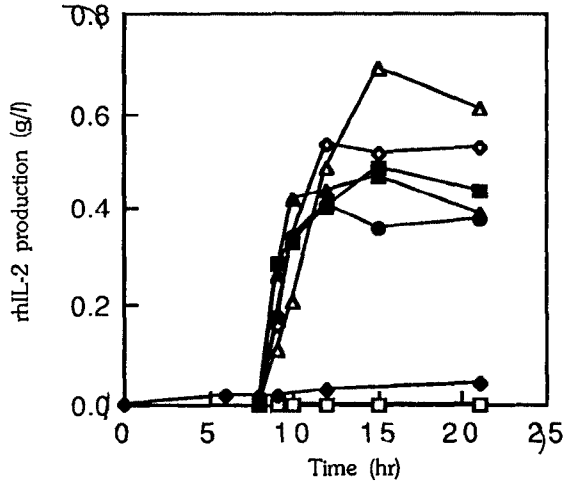

Fig. 7. Total rhIL-2 production on the basis of culture volume under various culture conditions.
Culture conditions are: (○) 34℃ (TS mode), (△) 36℃ (TS mode), (◇) 38℃ (TS mode), (●) 40℃ (TS mode), (■) 42℃ (TS mode), (▲) 44℃ (TS mode), (◆) 42℃ (CT mode).

Table 1. Effect of sonication intensity on cell lysate fractionation

Probe size	Number of sonication	Protein concentration (g/l)		Ratio of insoluble protein[1]	rhIL-2 content[2] (%)	Total rhIL-2[3] (g/l)
		insoluble fraction	soluble fraction			
Microprobe	1	1.70	0.61	0.74	31.2	0.53
	5	1.51	1.12	0.57	37.6	0.57
	10	1.23	1.38	0.47	44.9	0.55
Macroprobe	1	1.21	1.24	0.49	44.0	0.53
	5	0.91	1.50	0.38	56.9	0.54
	10	0.88	1.80	0.33	61.7	0.55

[1]Ratio of protein concentration in the insoluble fraction to the sum of insoluble and soluble fractions.

[2]rhIL-2 content in the insoluble protein fraction determined by SDS-PAGE analysis as described in Materials and Methods.

[3]Total rhIL-2 production in the culture estimated from the protein concentration in the insoluble fraction and the rhIL-2 content.

estimated from the data of cell viability and plasmid stability. The stability of recombinant cells is usually expressed as the ratio of plasmid-harboring cells to total viable cells. If one assumes that the plasmid-harboring cell fraction obtained by the replica plating method represents that of total cell populations in the reactor, then total plasmid-harboring population is simply equal to the number of viable cells multiplied by the plasmid-harboring fraction. The calculated results are shown in Fig. 5. A distinct reponse of recombinant cells to derepression of cloned-gene expression is evident. The number of plasmid-harboring cells under the derepressed condition was decreased rapidly since cell viability and plasmid stability are decreased upon induction. Increased deceleration of plasmid-harboring cell populations with increasing temperature can be observed.

β-Lactamase Activity

Since the ampicillin resistance gene (*bla*) and the rhIL-2 structural gene are located on the same plasmid, expression of *bla* gene might be competitive with the production of rhIL-2. Therefore, we have also investigated the effect of culture temperature on the expression of *bla* gene which was followed by the assay β-lactamase activity.

As shown in the sixth panel of Fig. 1, β-lactamase activity was reduced with increasing induction temperature. Highest β-lactamase activity was observed at cultivation temperature of 30℃, and at 42℃ (CT mode) the activity of β-lactamase was completely diminished. The loss of enzyme activity at 42℃ was not attributed to thermal inactivation (see Ref. 8). Instead, lower β-lactamase activity at higher induction temperature might be caused by the lowered plasmid-harboring cell populations following derepression.

As mentioned earlier, only plasmid-harboring cell populations should produce the gene product encoded in the plasmid. If this is the case, β-lactamase activity must be correlated with the number of plasmid harboring cells. Fig. 6(a) shows the relationship between β-lactamase activity and plasmid-harboring cells at 30℃ (CT mode). Fairly good correlation ($\gamma=0.993$) between these two parameters has been found. In Fig. 6(b) and (c), the data for temperature-shift experiments (36~44℃) are shown. To examine the difference in β-lactamase activity upon induction of rhIL-2 production the results were divided into two groups on the basis of induction time. The general trend for the data of repressed condition is, as expected, almost identical to that of Fig. 6(a). After the rhIL-2 induction, however, dependences of β-lactamase activity on the number of plasmid-harboring cells are rather different. It appears that the β-lactamase activity per plasmid-harboring cell is lowered with increasing induction temperature.

rhIL-2 Production

Since rhIL-2 was not detected in the soluble protein fraction, both rhIL-2 content and rhIL-2 production were examined on the basis of insoluble protein. As shown in the last panel of Fig. 1, the rhIL-2 expression was completely repressed at temperatures below 34℃. When the cultivation temperature was shifted to over 36℃, rhIL-2 gene expression was induced. The rhIL-2 content in the insoluble protein was highest at the induction temperature of 40℃. On the other hand, when recombinant cells were cultivated at 42℃ under constant-temperature mode, rhIL-2 content was very low as compared to that observed under temperature-shift mode at the same temperature. This result implies that the determination of an optimal induction time is also important for maximizing rhIL-2 production, as investigated in a previous study (7).

Many workers often used the cloned-gene product content, i.e. the percentage of product in the cellular protein, as a basis for comparison of the efficiency of recombinant protein synthesis. If this criterion is applied

to our case, the optimal induction temperature would be 40℃. However, the use of the product content for such a purpose may mislead the result. First, the product content can be varied with the experimental protocols. As mentioned earlier, rhIL-2 content was changed significantly depending on the sample preparation procedure, whereas total rhIL-2 production level per culture volume was nearly invariant (see Table 1). More importantly, the amount of product per culture volume is a true production level on a reactor basis. One should note that higher percentage of product in the cell does not ensure higher production in the culture for the reason that higher product formation usually accompanies the lowered cell growth and protein synthesis.

Therefore, we have examined total rhIL-2 production per unit culture volume determined by multiplying insoluble protein concentration by rhIL-2 content in the insoluble protein. Fig. 7 shows the results when the temperature was varied between 34℃ and 44℃. Highest rhIL-2 production was observed at the induction temperature of 36℃ instead of 40~42℃. This result appears to conflict with the previous study of Sugimoto *et al.* (22). They have reported that both production rate and final level of the cloned-gene product (β-galactosidase) were increased with increasing temperature in the range of 36℃ to 42℃, showing a maximum at 42℃. Cell growth was not affected by temperature in this range, although both cell growth and β-galactosidase production were inhibited above 43℃. The gene expression system they used was also composed of the cI857 repressor and P_L promoter.

A conflict between our result and Sugimoto's work can be explained based on the product formation kinetics proposed by one of the authors (9, 10). According to this model, the production rate of the cloned-gene product is roughly proportional to the efficiency of gene expression, ε, and the growth rate of plasmid-harboring cells, μ^+ (refer to Refs. 22, 23 for a complete description). Suppose that the dependence of the P_L promoter efficiency on culture temperature is approximately the same in two recombiant cell systems while the effect of culture temperature and/or product formation on the plasmid-harboring cell growth rate is different. If cell growth rate is unaffected by culture temperature as in the case of Sugimoto's work, then the optimum temperature which maximizes the cloned-gene product formation will be high. In the case where the product formation severely inhibits the cell growth (as observed in rhIL-2 production) optimum temperature will be lowered. Simulation results based on the kinetic model also indicate there exists an optimum gene expression efficiency for maximizing reactor productivity (10).

In the next, the effect of induction temperature on the efficiency of rhIL-2 production have been examined. The specific rhIL-2 production rate defined as the rate of rhIL-2 production per plasmid-harboring cell may be used as an approximate measurement of gene-expression efficiency. Table 2 shows that the degree of gene expression estimated in this way tends to increase at higher induction temperature, which agrees well qualitatively with a molecular-level study on the cI857 repressor-P_L promoter system (15).

In conclusion, as induction temperature was increased, the cell growth, recombinant cell viability, and plasmid stability of *E. coli* M5248[pNKM21] were adversely affected due to the stress on the cell metabolism while the expression of rhIL-2 gene was increased. One usually makes it a rule to induce the product formation at a temperature of 42℃ when the cI857 repressor-P_L promoter system is used for regulation of cloned-gene expression. We demonstrate here that the optimal temperature for maximizing target protein production (in our case 36℃) could be changed depending on the interaction between the host-cell metabolism and the cloned-gene expression.

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Table 2. Effect of induction temperature on the efficiency of rhIL-2 production

Induction temperature (℃)	Average number of plasmid-harboring cell[1] ($\times 10^{10}$)	rhIL-2 production rate[2] (g/l/hr)	Specific rhIL-2 production rate[3] (g/l/hr/10^{10} cells)
36	0.71	0.11	0.15
38	0.71	0.16	0.23
40	0.60	0.18	0.30
42	0.37	0.29	0.78
44	0.27	0.26	0.96

[1] arithmetic mean of the plasmid-harboring cell number during the first one hour after induction.
[2] rhIL-2 production rate during the first one hour after induction.
[3] rhIL-2 production rate per average number of plasmid-harboring cells.

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