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ORIGINAL ARTICLE

The role of *lac* operon and *lac* repressor in the induction using lactose for the expression of periplasmic human interferon-α2b by *Escherichia coli*

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Abstract The effect of *lac* operon in the induction using lactose for the expression of periplasmic human interferon-α2b (PrIFN-α2b) was studied in shake flask culture. *Escherichia coli* strains Rosetta2 (DE3) [R2 (DE3)] containing the *lac* operon and Rosetta-gami2 (DE3) [RG2 (DE3)] containing the deletion of entire *lac* operon with high level of *lac* repressor were used. R2 (DE3) over-expressed PrIFN-α2b at substantial levels (270–380 μg/L) in lactose-induced media. In spite of the deletion of *lac* operon in RG2 (DE3), the cells exposed to lactose produced PrIFN-α2b albeit in less quantity (18–20 μg/L). Under similar conditions, the percentage of IFN-α2b translocated into periplasm for cells induced with lactose was 43–57 and 15–30% in R2 (DE3) and RG2 (DE3), respectively. The PrIFN-α2b expressed by RG2 (DE3) grown in control medium and Terrific broth was 290.3 and 134.7 μg/L, respectively. The basal expression levels obtained in R2 (DE3) strain were 10-fold higher than those obtained in RG2 (DE3) strain. The target proteins expressed in uninduced state did not affect the growth, indicating that IFN-α2b was non-toxic to the bacterial cells. Equivalent level of PrIFN-α2b expression was obtained in RG2 (DE3) induced by IPTG and in R2 (DE3) induced by lactose.

Keywords Interferon-alpha2b · Lactose · IPTG · *lac* operon · *lac* repressor · *Escherichia coli* · Protein expression

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Introduction

Escherichia coli is often selected as the host for recombinant protein production because of its suitability for large-scale cultivation at reduced cost. The regulation of *E. coli lac* operon and its control elements has been one of the most widely studied in the aspect of molecular system since the 1960s (Grossman et al. 1998; Hansen et al. 1998; Jacob and Monod 1961; Kuhlman et al. 2007). pET vectors have been extensively applied for the expression of recombinant proteins in *E. coli* (Makrides 1996; Grossman et al. 1998; Ou et al. 2004). The gene of interest is placed under the control of T7 *lac* promoter in many of these vectors, and its expression is regulated by T7 RNA polymerase. Expression of recombinant protein can be induced by the addition of either lactose or a synthetic inducer into the recombinant bacterial culture. By utilizing the phenomenon of catabolite repression, autoinduction medium is designed for lactose-

inducible bacterial expression systems, in which protein expression is induced automatically from *lac* promoter without the need to monitor cell growth (Studier 2005).

Lactose is often used as an inducer for induction of T7 *lac* promoter due to low cost and because it is non-toxic to the bacterial cells. To initiate a transcription, lactose is transferred into cells by functional *lac* permease for active transport of lactose into bacteria (encoded by *lac*Y gene) (Mieschendahl et al. 1981). A portion of transported lactose is converted into allolactose by the β-galactosidase enzyme (encoded by *lac*Z gene) (Kalnins et al. 1983) to perform transgalactosidation reactions (Shukla and Wierzbicki 1975). β-galactoside transacetylase (encoded by *lac*A gene) is an enzyme that transfers an acetyl group from acetyl-CoA to β-galactosides and is responsible for lactose catabolism.

The analogue of lactose, isopropyl β-D-thiogalactoside (IPTG) is also widely used as an inducer. IPTG can be transfered into the cells by *lac* permease or by diffusion and then allosterically interacts with repressor molecules without any modifications (Mahoney 1998). Functional allolactose and IPTG, which bind to the *lac* repressor, will cause a structural change in the repressor resulting in loss in affinity for the operator. Thus, T7 RNA polymerase can bind to the promoter and transcribes the targeted gene. However, there is a drawback in a *lac* induction expression system where a problem of uninduced expression may occur. To overcome this problem, the pET system provided some strains with mutation or deletion of the *lac* operon with a high level of repressor production.

Lac control elements and repressors are important in protein synthesis (Blommel et al. 2007). A high level of the *lac* repressor ensures stringent repression in the uninduced state, but this approach may result in the elevated repression of T7 RNA polymerase synthesis. Transcription can be strongly repressed by the *lac* repressor, but T7 RNA polymerase initiates transcription very actively in the absence of the repressor, or in the presence of repressor plus inducer (Dubendorff and Studier 1991). Target protein expression levels in *lac*Y and *lac*Z mutant strains may become elevated when the strains are allowed to grow until the early stationary phase and the expression was greatly influenced by the medium composition and cell growth phase (Grossman et al. 1998).

Several induction approaches have been proposed for protein expression, which include manipulation of induction time (Neubauer et al. 1992; Vila et al. 1997), concentration of the inducer, and the composition of the growth medium (Donovan et al. 1996; Nancib et al. 1991). It is noted that evaluation of the effect of the *lac* operon on the expression level of IFN-α2b in the periplasmic region of *E. coli* using lactose has not previously been conducted. The objective of the present study was to determine the effect of the *lac* operon and *lac* repressor in the induction using lactose for the expression of periplasmic human interferon-α2b (PrIFN-α2b) by recombinant *E. coli*.

Materials and methods

Source of materials

Glucose, ammonium sulphate, magnesium sulphate, yeast extracts, K_2HPO_4, glycerol and Terrific broth were purchased from Merck, Germany. Peptone and lactose were purchased from Laboratorios Conda, Spain. Potassium phosphate was purchased from J.T. Baker, Mexico. Sodium phosphate was purchased from Sigma, Germany, while sodium chloride was purchased from Fisher Scientific, UK.

Characteristics of recombinant Rosetta2 (DE3) and Rosetta-gami2 (DE3)

Rosetta2 (DE3) [R2 (DE3)] host strain [F^- *ompT hsdSB*(rB^- mB^-) *gal dcm* (DE3) pRARE2] and Rosetta-gami 2 (DE3) [RG2 (DE3)] host strain [Δ(*ara*$^-$*leu*)7697 Δ*lac*X74 Δ*phoA Pvu*II *phoR araD139 ahpC galE galK rpsL* (DE3) F'[lac^+ $lacI^q$ *pro*] *gor*522::Tn10 *trx*B pRARE2] were used in this study. The main reason of choosing R2 (DE3) is to obtain more soluble product (not producing high level of repressors), to improve the recovery of intact recombinant proteins (lacking an outer membrane protease), and to reduce metabolic burden of the host cell (less formation of disulfide bonds in cytoplasm) according to its preferred genotypes. The main difference between these two strains is the *lac* operon for the transcription and the *lac* repressor for the basal expression. RG2 (DE3) contains a lactose mutant gene and has the deletion of the *lac* operon with a high level expression of the *lac* repressor while R2 (DE3) is a derivative from strain BL21 with the functional *lac* operon. Both strains carried seven codons rarely used in *E. coli*.

Preparation of recombinant R2 (DE3) strains

The details of the construction of the recombinant pET26b-IFNα2b plasmid and RG2 (DE3) strain have been described in our previous study (Ramanan et al. 2010b). The expression vector which contains T7 *lac* promoter, pelB signal sequence and IFN-α2b gene was transformed into R2 (DE3) competent cells, to obtain the recombinant strain (R2 (DE3)/pET26b-IFNα2b) for the production of the target protein. R2 (DE3) and RG 2(DE3) have similar expression hosts, containing the plasmid that carries the genes for seven codons, rarely used in *E. coli*. This approach can be used to enhance the expression level of heterologous proteins by overcoming codon bias (Schumann and Ferreira 2004;

Sørensen and Mortensen 2005). Additionally, R2 (DE3) contains a lamda prophage which has the gene for T7 RNA polymerase.

Fermentations

The stock cultures of R2 (DE3) and RG2 (DE3) were cultivated at 37°C until an early log phase was reached (OD=0.4–0.6). The cells were revived by modulating 1 mL of stock culture into 50 mL TB medium supplemented with 30 mg/L kanamycin and 34 mg/L chloramphenicol. The culture was grown at 37°C with a constant shaking at 250 rpm for 16 h. To start the fermentation, 8% (v/v) of inoculum was added into 250-mL baffled shake flasks containing 50 mL of sterilized production medium. The flasks were incubated at 37°C in an incubator shaker (Certomat® BS-1 B; Braun, Germany), agitated at 250 rpm. The cultures were induced with lactose (0.2–4%) or IPTG (1 mM) for different periods of cultivation according to the need of each experiment, and then the temperature was switched to 30°C. Samples were withdrawn at timed intervals after the induction for analysis.

Medium and induction

The optimized medium for the expression of PrIFN-α2b using IPTG by recombinant *E. coli* as proposed by Tan et al. (2009) was used in this study for lactose-induced culture. This medium consisted of 5.5 g/L glucose, 10 mL/L glycerol, 55.2 g/L yeast extract and 42.3 g/L peptone, and 10 mL/L of 0.1 M of potassium buffer. Initial pH of the medium was adjusted to 7 using either 1 M HCl or 1 N NaOH. To study the effect of lactose as an inducer in the optimized medium, 4% (w/v) lactose (LacM) was added to the medium at 4 h of fermentation to replace 1 mM IPTG. Fermentations using IPTG as an inducer were also carried out as controls. For comparison, medium formulations as reported in the literature such as ZYP-5052 (Studier 2005) and Kotik (Kotik et al. 2004) were modified and also tested for the induction of PrIFN-α2b by lactose. The modified ZYP-5052 medium consisted of 10 g/L casamino acid, 5 g/L yeast extract, 50 mM disodiumphosphate, 50 mM potassium diphosphate, 2.5 M ammonium sulphate, 50 mM magnesium sulphate, 5 g/L glycerol, 0.5 g/L glucose, and 2 g/L lactose. The modified Kotik medium consisted of 13 g/L glycerol, 18 g/L peptone casein, 3 g/L lactose, 14.6 g/L di-sodium phosphate, 3 g/L potassium diphosphate, 0.5 g/L sodium chloride, 1 g/L ammonium sulphate, and 0.25 g/L magnesium sulphate.

Analytical methods

Cell concentration was quantified by optical density (OD) and dry cell weight (DCW). The OD was measured at 600 nm using spectrophotometer (Lambda 25; Perkin Elmer). The relationship between DCW and OD_{600} can be expressed as; DCW (g/L)=3.59 OD_{600}.

The culture sample was centrifuged (rotor model 1619, Universal 32R centrifuge; Hettich, Switzerland) at 8,000*g* for 10 min at 4°C. The supernatant was used for glucose analysis and the cell pellet was disrupted for the extraction of PrIFN-α2b using the osmotic shock method as described by Ramanan et al. (2009). The shrunk cells after osmotic shock extraction were disrupted with glass bead shaking as described elsewhere (Ramanan et al. 2008) for 25 min. The disrupted cells were then centrifuged at 8,000*g* for 10 min at 4°C, where the supernatant was used for the determination of soluble cytoplasmic IFN-α2b (cIFN-α2b). Sodium dodecyl sulphate (SDS) (0.05%) was added to the cell pellet for 5 min and extracted as inclusion bodies IFN-α2b (IbIFN-α2b).

The concentration of glucose was analyzed using Biochemistry Analyzer (Model 2700 Select; YSI). The quantity of IFN-α2b was determined using the fully automated Surface Plasmon Resonance detection system (BIAcore 3000; GE HealthCare) according to the method described by Ramanan et al. (2010a). In brief, the supernatant extracted was mixed with HBS-EP buffer in 1:1 ratio and the resultant solution was passed to the reference and anti-IFN flow cells for 1 min at a flow rate of 5 μL/min with a measurement unit of Response Unit (RU). After each measurement, the surface was regenerated with a 1-min pulse of 10 mM glycine (pH 2.5) at 5 μL/min. The relationship between IFN-α2b and RU can be expressed as; IFN-α2b (μg/L)=0.363 RU. All experiments were carried out at 25°C with the CM5 chip and the results were further analyzed using BIA evaluation software (v.4.1).

Results

Effect of the *lac* operon on protein expression and translocation of soluble IFN-α2b to the periplasmic space of *E. coli*

A series of media with different nutrient constituents were used to cultivate R2 (DE3) and RG2 (DE3) to perform the subsequent induction phase aimed to achieve high levels of PrIFNα2b expression using IPTG and lactose as the inducer molecules (Table 1). R2 (DE3) expressed significant levels of PrIFN-α2b (267–375 μg/L) in all lactose- and IPTG-induced media. The *lac* mutant strain RG2 (DE3) expressed PrIFN-α2b at a very low level when lactose was used as inducer, where the maximum expression level was 20.4 μg/L, and this gave the specific yield of only 2.7 μg/g DCW. When IPTG was used as an inducer, RG2 (DE3) expressed

Table 1 Effect of different media and inducers on growth of RG2 (DE3) and R2 (DE3) and their ability to produce IFN-α2b

Media		Time (h)	Inducer	DCW (g/L)	PrIFN-α2b (μg/L)	TIFN-α2b (μg/L)	Specific yield (μg/g cell)	Volumetric productivity (μg/L/h)	Transfer (%)
Control	S_1	8	IPTG	7.10 (0.14)	290.3 (0.60)	496.8 (0.51)	40.9	36.3	58.4
	S_2	6	IPTG	8.02 (0.39)	364.8 (0.23)	919.4 (1.23)	45.5	60.8	39.7
TB	S_1	8	IPTG	6.28 (0.04)	134.7 (0.06)	234 (0.21)	21.4	16.8	57.6
	S_2	6	IPTG	9.27 (1.66)	267.5 (0.97)	544.6 (0.47)	28.9	44.6	49.1
LacM	S_1	14	Lactose	7.46 (0.23)	20.4 (0.28)	133.7 (0.15)	2.7	1.5	15.3
	S_2	16	Lactose	8.01 (1.35)	275.3 (1.40)	634.9 (0.83)	34.4	17.2	43.4
ZYP	S_1	14	Lactose	7.16 (0.07)	19.9 (0.43)	64.6 (0.02)	2.8	1.4	30.8
	S_2	16	Lactose	10.27 (2.04)	374.5 (0.61)	677.6 (0.78)	36.5	23.4	55.3
Kotik	S_1	14	Lactose	10.72 (0.17)	18.2 (0.05)	62.2 (0.08)	1.7	1.3	29.3
	S_2	24	Lactose	17.13 (2.97)	325.8 (1.88)	571.8 (0.29)	19.0	13.6	57.0

The results of final cell concentration and final PrIFN-α2b and TIFN-α2b [PrIFN-α2b (periplasmic)+cIFN-α2b (soluble cytoplasmic)+IbIFN-α2b (inclusion bodies)] are the average of triplicate experiments. Values in parentheses are standard deviations. Specific yields and volumetric productivity are calculated with the average values

S_1 RG2 (DE3), S_2 R2 (DE3)

PrIFN-α2b up to a level of 290.3 μg/L, which was ten times higher than that obtained when lactose was used. Although the total IFN-α2b expression level in R2 (DE3) was two-fold higher than the RG2 (DE3) cells in the control medium, the periplasmic secretion was only slightly increased (1.26-fold). In terms of specific yield, the value obtained by RG2 (DE3) in IPTG-induced culture (40.9 μg/g DCW in control medium) was about 13 times higher than the values for lactose-induced culture (2.7 μg/g DCW in LacM medium). In lactose-induced culture, the percentage of IFN-α2b translocated into periplasmic region by R2 (DE3) was ranged from 43 to 57%, while the IFN-α2b secreted in periplasmic space was greatly reduced to 15–30% in RG2 (DE3). However, the secretion rate of RG2 (DE3) was increased by 2-fold (58%) when IPTG was used as an inducer.

The role of lactose in retaining the expression of target gene

The growth of both R2 (DE3) and RG2 (DE3) was higher in lactose-induced media as compared to IPTG-induced media (Fig. 1). In both strains, the expression of PrIFN-α2b ceased markedly over time after 4 h of induction by IPTG in both terrific broth and optimized medium. In contrast, the expression of PrIFN-α2b was retained for a longer duration in R2 (DE3) culture induced with lactose. In ZYP-5052 medium, PrIFN-α2b only began to decrease at a slow rate after the maximum expression was achieved at 16 h of fermentation. In Kotik medium, the expression of PrIFN-2b was increased up to 24 h of fermentation. However, PrIFN-α2b was not significantly expressed (18–20 μg/L) in RG2 (DE3) culture

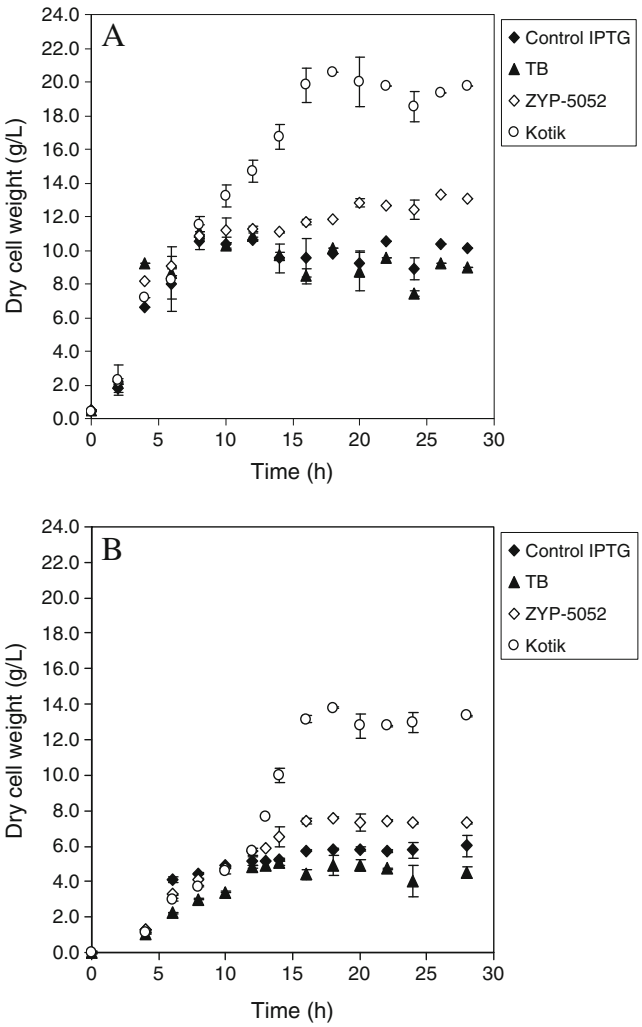

Fig. 1 Growth profile, measured as dry cell weight, of **a** R2 (DE3) and **b** RG2 (DE3) in different media of fermentation

induced using lactose as compared to R2 (DE3) culture due to the Δ(*lac*)X74 and *lac* genetic marker which signifies the deletion of the *lac* operon from the chromosome and unable to utilize lactose.

Basal expression

Basal expressions of PrIFN-α2b for both the strains were analyzed at 4 h of fermentation prior to IPTG induction (Fig. 2). The expression of PrIFN-α2b (range 150–200 μg/L) by R2 (DE3) occurred at the early stages of fermentation using all media, prior to induction. This is contrary to the result in which very low basal expression (10–30 μg/L) of target genes was achieved in RG2 (DE3), but the usual high levels of expression were obtained upon induction. The basal expression rate of PrIFN-α2b in R2 (DE3) was approximately 10-fold higher than that obtained in RG2 (DE3), which contains the *lac*I^q genotype (the gene responsible for producing high levels of *lac* repressor) in suppressing the basal expression.

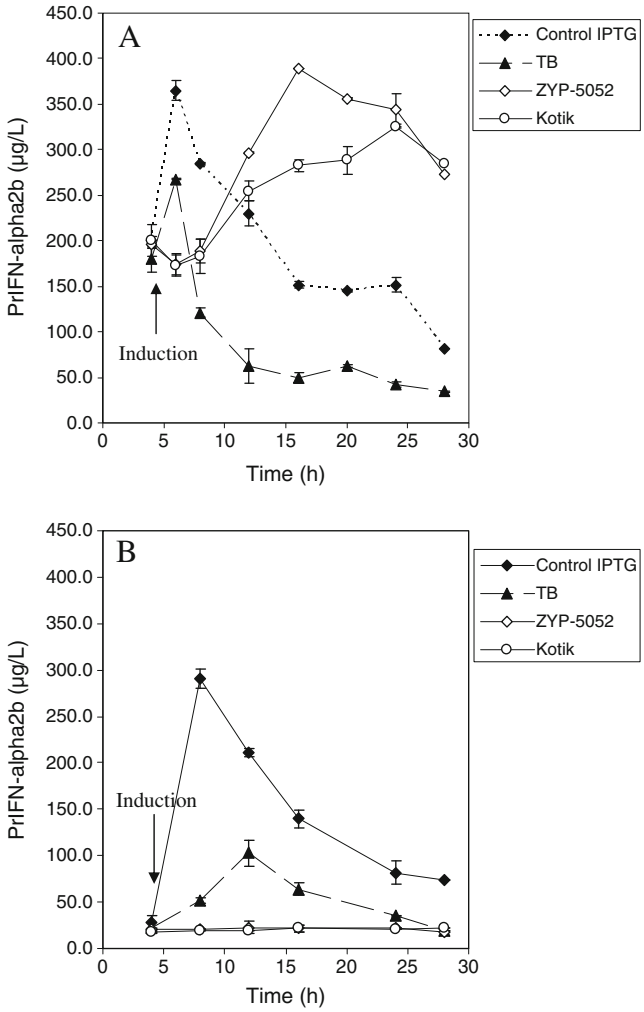

Fig. 2 Expression of PrIFN-α2b during the cultivation of **a** R2 (DE3) and **b** RG2 (DE3) in different media

Effect of lactose concentration on the expression of PrIFN-α2b

The concentration of lactose in ZYP-5052 medium was modified according to the design of experiment at a range from 0.2–4% (w/v) for the R2 (DE3) strain. With pET26b–IFNα2b as the expression vector, the periplasmic protein expression level was triggered by adding different concentrations of lactose to the culture. The expression of PrIFN-α2b was gradually increased from 20 to 25% with increasing lactose concentration from 0.2 to 4% (w/v) during induction (Table 2). However, the concentration of lactose did not exert a significant influence on the growth of *E. coli* in ZYP-5052 medium. The total expression of IFN-α2b increased gradually with increasing lactose concentration up to 4% (w/v), which was similar to the profile of the expression of PrIFN-α2b. Maximum expressions of PrIFN-α2b (413.0 μg/L) and cIFN-α2b (612.2 μg/L) were obtained when 4% (w/v) lactose was used as an inducer.

The effect of induction point on the expression by *lac* operon

The effect of the induction point was evaluated by adding 4% lactose at the exponential growth phase (4 h induction point) and at the stationary phase (12 h induction point). Results of the growth of *E. coli* and the expression of PrIFN-α2b in ZYP-5052 medium induced with lactose at different induction points are shown in Table 3. In all fermentations, glucose was exhausted when the exponential growth phase was achieved (data not shown). For 12 h induction, growth of R2 (DE3) was abruptly arrested at DCW of 8.07 g/L and reached a stationary growth phase after 8 h of fermentation. On the other hand, R2 (DE3) continued to grow markedly for 4 h induction. The growth of RG2 (DE3) was about 10% higher at 4 h induction as compared to 12 h induction, yet both induction phases yielded similar level of PrIFN-α2b (10–20 μg/L). With different induction points, the highest value of PrIFN-α2b (380.7 μg/L) was observed in R2 (DE3) when induced with lactose at the middle of exponential growth phase, where the translocation rate reached its maximum value (55.7 %). PrIFN-α2b decreased rapidly when lactose was added at a stationary phase (107.7 μg/L), suggesting that induction point was the important factor for efficient expression of periplasmic protein in *E. coli*. A very low percentage of soluble IFN-α2b translocated to the periplasmic space was observed (13.6 and 16.5% for induction point at 4 and 12 h, respectively) when RG2 (DE3) was induced with lactose.

Table 2 Effect of different concentrations of inducers in ZYP-5052 medium on growth of R2 (DE3) and its ability to produce IFN-α2b

Lactose concentration (%)	DCW (g/L)	PrIFN-α2b (periplasmic) (μg/L)	TIFN-α2b (total) (μg/L)	Specific yield (μg g^{-1} cell)	Volumetric productivity (μg/L/h)	Transfer (%)
0.2	10.89 (0.64)	334.3 (10.19)	557.1 (5.40)	30.7	41.8	60.0
0.5	9.82 (0.23)	326.6 (1.42)	533.1 (4.97)	33.3	54.4	61.3
1.0	10.40 (0.19)	361.4 (1.91)	567.7 (7.35)	34.8	45.2	63.7
2.0	13.11 (0.15)	390.3 (3.69)	595.3 (5.97)	29.8	65.1	65.6
4.0	12.42 (0.17)	413.0 (5.20)	612.2 (6.12)	33.3	29.5	67.5

The results of final growth of R2 (DE3), final PrIFN-α2b and TIFN-α2b [PrIFN-α2b (periplasmic)+cIFN-α2b (soluble cytoplasmic)+IbIFN-α2b (inclusion bodies)] are the average of triplicate experiments. Values in parentheses are standard deviations. Fermentation time was 16 h

Discussion

The expression of PrIFN-α2b can be regulated by lactose to a significant level by controlling the expression of the *lac* operon gene to regulate its rate of transcription. In the absence of the *lac* operon, the expression of PrIFN-α2b cannot be regulated by lactose to a significant level, and failure in the *lac* operon induction process resulted in significant reduction in the formation of allolactose, the true inducer of the *lac* operon. This was presumably due to a defect in lactose accumulation (Flagg and Wilson 1976). Therefore, the present study was performed to strengthen the above observation by examining to what extent the *lac* operon would affect recombinant IFN-α2b production in periplasm.

Basal expression can be controlled in an inducible T7 expression system by blocking the target T7 promoter with the *lac* repressor (Dubendorff and Studier 1991). The availability of the *lacI*^{q} gene in RG2 (DE3) encodes high levels of repressor that exhibits tight regulation of the *lac* promoter, and this high level repressor ensures stringent repression in the uninduced state. The presence of high levels of *lac* repressor in RG2 (DE3) strongly inhibited the transcription by the *lac* operator (Cooper and Magasanik 1974). However, when lactose or IPTG is added to the culture medium, it caused the repressor to be released from the operator to enable RNA polymerase to initiate the transcription (Reznikoff and Miller 1978; Dubendorff and Studier 1991). In R2 (DE3) cultures, basal expression did not affect the cell growth and expression of PrIFN-α2b. This shows that the IFN-α2b expressed by these strains did not exert toxicity on the cells during the fermentation.

With the deletion of the *lac* operon, lactose is not effectively utilized as inducer, which resulted in a high level of the repressor molecule in RG2 (DE3) bound to upstream *cis* activated operator and reduced the expression of T7 RNA polymerase (Schumann and Ferreira 2004). Unlike lactose, IPTG is not metabolized by the cell (Donovan et al. 1996), but transported into the cell by methods other than *lac* permease and hence directly bound to the *lac* repressor reducing its affinity for the operator (Mahoney 1998; Lewis 2005). This action enhanced the synthesis of T7 RNA polymerase and subsequently induced the transcription and translation of IFN-α2b in RG2 (DE3).

A high concentration of inducer would increase the level of transcription in the cells, thus enhancing the expression and/or translocation of some secreted proteins (Donovan et al. 1996). However, a higher rate of expression would affect the rate of translocation in periplasmic expression (Rosenberg 1998), and the excess of expressed recombinant protein is likely to accumulate in inclusion bodies (Mergulhao et

Table 3 Effect of induction point in ZYP-5052 medium induced by lactose on growth of R2 (DE3) and RG2 (DE3) and their ability to produce IFN-α2b

	RG2 (DE3)		R2 (DE3)	
	Induction point		Induction point	
	4 h	12 h	4 h	12 h
Fermentation time (h)	8	16	8	16
Final DCW (g/L)	7.52 (0.91)	6.82 (0.07)	11.02 (0.52)	8.07 (0.83)
Final glucose (g/L)	0.052 (0.05)	0.046 (0.09)	0.037 (0.03)	0.021 (0.07)
Final PrIFN-α2b (μg/L)	10.4 (0.17)	21.8 (0.64)	380.7 (6.56)	107.7 (5.32)
Final IbIFN-α2b (μg/L)	35.8 (0.08)	40.2 (8.01)	50.4 (1.23)	67.3 (6.42)
Final cIFN-α2b (μg/L)	30.3 (2.67)	70.2 (1.04)	250.9 (4.22)	169.1 (1.46)
Transfer (%)	13.6	16.5	55.7	31.3

The results of final PrIFN-α2b (periplasmic), IbIFN-α2b (inclusion bodies) and cIFN-α2b (soluble cytoplasmic) are the average of triplicate experiments. Values in parentheses are standard deviations

al. 2005; Mergulhão and Monteiro 2007). Thus, some soluble recombinant proteins were expressed in high levels using lower concentrations of IPTG as inducer (Azaman et al. 2010). On the other hand, the rate of translocation was also reduced in the cultures expressing a lesser amount of soluble IFN-α2b probably due to the competition of periplasmic host proteins. It should be noted that the high level of periplasmic expression could be achieved in a narrow range of IPTG inducer concentration where the rate of production should match the rate of translocation of the protein (Azaman et al. 2010). In the case of lactose, the increase in the amount of lactose did not greatly influence expression of PrIFN-α2b (Table 2). As the lactose could be used as a carbon source for cell metabolism other than the inducer, lactose provides flexibility of choosing the preferred level of concentration without having any deleterious effect on the translocation of expressed protein.

Leakage of periplasmic protein can occur during cell division, auto cell lysis (Somerville et al. 1994) and due to the accumulation of recombinant protein in the periplasm. In the last case, the accumulation of protein in periplasm would increase the osmotic pressure and hence drive the protein across the outer membrane (Hasenwinkle et al. 1997). Additionally, perturbations in the membrane (Pugsley et al. 1997) occurred during the production of proteins and thus increased its selective permeability (Slos et al. 1994). Leakage of protein might also occur due to the use of IPTG as inducer which would cause the metabolic burden to the cell (Kilikian et al. 2000; Baneyx 1999). For example, leakage of active subtilisin E in *E. coli* into the culture occurred at 6 h after induction with IPTG (Takagi et al. 1988). In our case, a drastic decrease in PrIFN-α2b level could also be due to the leakage of protein into the culture or due to the degradation of protein in prolonged fermentation induced with IPTG. It is important to note that this phenomenon was not observed in lactose-induced culture. Lactose appeared to enhance the solubility and retained the secreted proteins and benefited greatly from the lower transcription rates as compared to IPTG.

In the process of recombinant protein production by *E. coli*, lactose induction is a switching point between cell growth and recombinant protein synthesis. The transcription of the foreign gene on the plasmid begins with the addition of lactose and in consequence brings great changes to the metabolism of the host cell by initiating the translation of heterologous protein. With the presence of glucose at the beginning of fermentation, there is no induction of the *lac* operon by lactose as growth on glucose causes inducer exclusion, which means that lactose is unable to enter the cell. This situation occurred due the potentiation of growth on glucose, which is inhibitory to *lac*Y transport and for other sugar transport systems (Osumi and Saier 1982). Growth on glucose also reduced the levels of cAMP in the cells, which in turn reduced the ability to induce the expression of the *lac* operon. cAMP promoted the transcription of the *lac* operon by binding to CAP (catabolite gene activator protein) and caused an allosteric transformation (Lee et al. 2005; Wong et al. 1997). Lactose added after 4 h of fermentation in R2 (DE3) was immediately utilized for cell maintenance and protein expression after the exhaustion of the primary carbon source due to catabolite repression (Magasanik 1961). When the culture was induced at a stationary growth phase, R2 (DE3) was unable to express PrIFN-α2b at high levels. Reduced expression was mainly due to glucose starvation which abruptly stopped RNA accumulation in the cells (Chaloner-Larsson and Yamazaki 1978), and subsequently repressed the expression of PrIFN-α2b. The availability of glucose in the growth medium had no impact on the expression of PrIFN-α2b in by RG2 (DE3) as lactose was incapable of inducing the T7*lac* promoter. The expression of PrIFN-α2b by R2 (DE3) obtained at the 4 h induction point was significantly higher than that obtained at 12 h induction when lactose was used as an inducer. This is because the nutrient required for gene expression is more than sufficient in the earlier stages as compared to the later stages of fermentation (Donovan et al. 1996). Results from this study indicate that the selection of an appropriate induction time greatly influenced the *lac* operon induction, which subsequently affected the expression of PrIFN-α2b.

In this study, the availability of the *lac*I^q gene in RG2 (DE3) encoded high levels of repressor that ensures stringent repression in the uninduced state. The basal expression in R2 (DE3) was shown to be non-toxic towards *E. coli* cells. In addition, lactose is a cheap inducer and does not exert a toxic effect on the cells which may affect cell growth and expression of the proteins. Thus, it would be advantageous to use lactose as inducer to obtain the same level of transcription as IPTG.

Conclusion

In spite of the deletion of the *lac* operon, a low level of expression of IFN-α2b by RG2 (DE3) was observed in the lactose-induced cultures. On the other hand, R2 (DE3) overexpressed PrIFN-α2b at substantial levels (270–410 μg/L) in lactose-induced media. Lactose-induced culture greatly enhanced (~18 times) the expression of PrIFN-α2b in the presence of the *lac* operon gene. The percentage of IFN-α2b translocated into periplasm induced with lactose was 43–67% in R2 (DE3). The basal expression levels obtained in R2 (DE3) strain were 10-fold higher than those obtained in RG2 (DE3) strain. Lactose induction in R2 (DE3) has an equivalent amount of expression to RG2 (DE3) induced by IPTG. In addition, maximum production of PrIFN-α2b

(380.7 μg/L) was observed in R2 (DE3) when induced by lactose at the middle of the exponential growth phase. Thus, selection of the host strain via suitable genetic markers is essential in the induction of lactose for expression of the target gene.

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