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Characterization of the T7 promoter system for expressing penicillin acylase in *Escherichia coli*

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Abstract The *pac* gene encoding penicillin acylase (PAC) was overexpressed under the regulation of the T7 promoter in *Escherichia coli*. PAC, with its complex formation mechanism, serves as a unique target protein for demonstration of several key strategies for enhancing recombinant protein production. The current T7 system for *pac* overexpression was fraught with various technical hurdles. Upon the induction with a conventional inducer of isopropyl-β-D-thiogalactopyranoside (IPTG), the production of PAC was limited by the accumulation of PAC precursors (proPAC) as inclusion bodies and various negative cellular responses such as growth inhibition and cell lysis. The expression performance could be improved by the coexpression of *degP* encoding a periplasmic protein with protease and chaperone activities. In addition to IPTG, arabinose was shown to be another effective inducer. Interestingly, arabinose not only induced the current T7 promoter system for *pac* expression but also facilitated the posttranslational processing of proPAC for maturation, resulting in significant enhancement for the production of PAC. Glycerol appeared to have an effect similar to, but not as significant as, arabinose for enhancing the production of PAC. The study highlights the importance of developing suitable genetically engineered strains with culture conditions for enhancing recombinant protein production in *E. coli*.

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Introduction

Due to the well-characterized genome and techniques available for genetic manipulation, *Escherichia coli* remains the most common host system as a protein overproducer for industrial applications. Biochemical and genetic engineering strategies have been developed toward high-level gene expression (Andersen and Krummen 2002; Baneyx 1999; Makrides 1996). The most effective means is to boost gene expression at a transcriptional level via strong promoter systems. Among them, the *lac* promoter and its derivatives, such as *lacUV5*, *tac*, and *trc*, inducible by lactose or its analog, IPTG, had been adopted extensively (Amann et al. 1988; DeBoer et al. 1983; Yanisch-Perron et al. 1985). Another promoter system, also considered to be a *lac* derivative, is T7, in which RNA polymerase from bacteriophage T7 is expressed out of the host chromosome under the regulation of the *lacUV5* promoter and is then bound to the T7 promoter for efficient transcription of the target gene on the plasmid (Studier and Moffatt 1986). Other promoter systems with various induction factors such as chemicals [e.g., arabinose (Guzman et al. 1995) or tetracycline (Lutz and Bujard 1997)], heat (Remaut et al. 1981), pH (Chou et al. 1995), the availability of oxygen (Khosla et al. 1990) or carbon source (Tunner and Robertson 1992), etc., had been developed. With this approach, accumulation of intracellular recombinant proteins at a high level up to 40–50% of the total cellular protein becomes achievable.

The formation of inclusion bodies represents one of the major technical hurdles in bioindustry for recombinant protein production (Villaverde and Carrio 2003). One of the reasons for inclusion body formation is that the overex-

pressed gene products cannot be suitably processed by folding modulators to generate an appropriate protein structure (Baneyx and Mujacic 2004). For extracytoplasmic proteins, the efficiencies for translocation, posttranslational folding, processing, and targeting become important as well. In principle, the precursor, intermediate, or final gene product can possibly form inclusion bodies in the cytoplasm and/or periplasm. This brings up a technical issue that, in addition to improving the efficiency of each gene expression step (i.e., transcription, translation, and posttranslational steps), a "balanced" protein synthesis flux throughout these steps should be properly maintained to avoid the accumulation of any species along the protein formation pathway.

Another key issue for recombinant protein production is the physiological burden caused by gene overexpression, frequently reflected by a number of negative cellular responses such as growth inhibition, cell lysis, or even death (Dong et al. 1995; Kurland and Dong 1996). The deterioration in cell physiology, along with various negative cellular responses, tends to reduce the yield of recombinant protein. Genetic manipulation for improving cell physiology has been exploited to enhance recombinant protein production (Chou et al. 1996; Schweder et al. 2002; Vila et al. 1997). Studies have also been conducted to characterize the cellular responses to recombinant protein overproduction (Gill et al. 2000; Oh and Liao 2000). Such fundamental knowledge would be valuable in developing systematic and theoretical approaches to enhance recombinant protein production.

In this study, the *E. colipac* gene encoding penicillin acylase (PAC), a key industrial enzyme for the production of several β-lactam antibiotics (Elander 2003; Shewale and Sivaraman 1989), was overexpressed under the regulation of the T7 promoter. Formation of mature PAC in the periplasm of *E. coli* involves a series of posttranslational steps, including translocation and periplasmic processing/folding steps, which are unique for prokaryotic proteins (Sizmann et al. 1990). The periplasmic processing mechanism consists of various proteolytic steps, including the intramolecular autoproteolysis for the first cleavage of PAC precursor (proPAC) at the junction between the connecting peptide and β subunit and the subsequent proteolyses for chopping the connecting peptide (Kasche et al. 1999; Sizmann et al. 1990). While *E. coli pac* was previously overexpressed under the regulation of *lac*-derived promoters (Chou et al. 1999; Scherrer et al. 1994; Sriubolmas et al. 1997), the use of the T7 promoter has not been explored. We have demonstrated that, in addition to a conventional inducer of IPTG, arabinose can serve as another effective inducer for induction of the current T7 promoter system for *pac* overexpression. The formation of periplasmic inclusion bodies, primarily composed of proPAC, and the toxicity of gene products, resulting in severe growth inhibition and cell lysis, were identified as key obstacles for *pac* overexpression regulated by the *trc* promoter (Chou et al. 1999), and the bottlenecks can be eliminated through the presence of exogenous DegP, a periplasmic protein with protease and chaperone activities (Pan et al. 2003). These technical issues for the production of PAC also existed in the current T7 system and could be resolved through either arabinose induction or *degP* coexpression.

Materials and methods

JM109(DE3) and BL21(DE3) were used as the host for the production of recombinant PAC. Molecular cloning was performed according to standard protocols (Sambrook et al. 1989), and HB101 was the host for cloning. Restriction enzymes were purchased from New England Biolabs (Beverly, MA, USA). Polymerase chain reaction (PCR) was conducted in an automated thermal cycler (Amplitron II, Thermolyne, Dubuque, IA, USA). Purification of plasmid DNA was performed using a spin-column kit purchased from Clontech (Palo Alto, CA, USA). Plasmid transformation was carried out using an electroporator (*E. coli* Pulser, Bio-Rad, Hercules, CA, USA) or a chemical method according to Chung and Miller (1993). The *E. coli pac* gene was amplified by PCR with *pfu* DNA polymerase (Stratagene, La Jolla, CA, USA) as the PCR polymerase, PK1/PK3 (5′-CCTGCCAGAGGAT*CATATG* AAAAATAGAA-3′/5′-CCAGAATATGAGG*GAATTC*GGG CTT-3′, where the restriction sites are underscored and the mutations are in italics) as the primer pair, and pCLL2902 (Chou et al. 1999) as the template. The plasmid of pET-20b(+) (Novogene, Madison, WI, USA) was used as the backbone of the *pac* expression vector. Because pET-20b (+) contains the *bla* gene, whose gene product of β-lactamase tends to attack the β-lactam bond of penicillin and therefore affect PAC enzyme assay, the *bla* gene was replaced with a Kn-resistant (Kn^R) gene before inserting the *pac* gene. To do this, pACYC177 (Chang and Cohen 1978) was first amplified in GM48, a *dcm* mutant (Marinus 1973), to avoid the Dcm methylation at the two *Stu*I sites. The purified plasmid was cleaved with *Stu*I, and the 1.3-kb DNA fragment containing the Kn^R gene was gel-extracted. pET-20b(+) was digested with *Dra*I, and the resulting largest DNA fragment at 3.0 kb was gel-extracted and ligated with the 1.3-kb Kn^R cassette to form pETKn-20b(+). The above PCR product flanked with *Nde*I and *EcoR*I at 2.8 kb was purified and inserted into the corresponding restriction sites of pETKn-20b(+) to form a transcriptional fusion of pETKn29a. Therefore, pETKn29a contains the *E. coli* wild-type *pac* gene, encoding preproPAC (i.e., S + α + C + β, where S, α, C, and β represent signal peptide, α subunit, connecting peptide, and β subunit, respectively), the expression of which is under the regulation of the T7 promoter. PAC-producing strains can be identified with a microbiological screening protocol (Meevootisom et al. 1983). pARDegP contains the *E. coli degP* gene fused with the *araB* promoter (Pan et al. 2003). pETKn29a has a pBR322 replication origin and a Kn^R marker and is, therefore, compatible with pARDegP having a pACYC184 replication origin and a

chloramphenicol-resistant (Cm^R) marker. All of the experimental operations, including bacterial cultivation, culture sample processing, PAC assay, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and Western blotting were conducted according to the protocols previously described (Xu et al. 2005).

Results

Effect of inducer (IPTG)

The use of a conventional inducer of IPTG for induction of *pac* expression regulated by the T7 promoter system was first explored, and the results are summarized in Table 1 and Fig. 1. With JM109(DE3) as the host, *pac* expression was highly induced by IPTG. However, most of the induced gene products were in an insoluble form, with proPAC as the major species. Compared to the control experiment without adding the inducer, the specific PAC activity (i.e., from mature PAC) was induced at approximately sixfold (e.g., 1A3 vs 1A1). Due to the physiological burden, cell growth was severely inhibited upon *pac* overexpression (e.g., 1A3 vs 1A1), and a large amount of cells were lysed as most of the PAC activity was detected extracellularly (e.g., PAC release levels of 75 and 81% for 1A4 and 1A5, respectively). On the other hand, with BL21(DE3) as the host, the expression of *pac* was also IPTG-inducible. Although the specific PAC activity was highly induced up to 20-fold compared to the control experiment, such *pac* expression level was much lower than that of recombinant JM109(DE3) under the same culture conditions. The induced gene products, also with proPAC as the major constituent, remained in both soluble and insoluble forms in approximately equal amounts. Unlike JM109(DE3), there was neither growth inhibition nor cell lysis upon *pac* overexpression for recombinant BL21(DE3), even at a relatively high IPTG concentration of 0.5 mM, and most of PAC activity remained intracellular (e.g., PAC release levels of 13 and 10% for 1A8 and 1A9, respectively).

Effect of inducer (arabinose)

Recently, arabinose was shown to be able to induce leaderless *pac* (LL *pac*) expression regulated by the *trc* promoter in *E. coli* (Xu et al. 2005). The T7 promoter system is therefore expected to be arabinose-inducible as the expression of the bacteriophage T7 gene 1, encoding T7 RNA polymerase, out of the host chromosome is under the regulation of the *lacUV5* promoter. The results of arabinose induction of *pac* expression for the T7 promoter system are summarized in Table 1 and Fig. 1. With JM109 (DE3) as the host, *pac* expression was highly inducible by arabinose. Most of the induced gene products remained in a soluble form. The amount of inclusion bodies for arabinose-induced cultures remained at a much lower level compared to that for IPTG-induced cultures (e.g., 1A8 vs 1A4) and generally increased with the arabinose concentration (i.e. 1A6–1A9). The specific PAC activity was induced up to a high level of approximately tenfold (e.g., 1A9 vs 1A1). On the other hand, with BL21(DE3) as the host, *pac* expression was also arabinose-inducible, but PAC activity was lower than that of recombinant JM109(DE3) under the same culture conditions. For both hosts, there was neither growth inhibition nor cell lysis upon arabinose induction for *pac* expression, and most of PAC activity remained intracellular. In addition, the amount of proPAC inclusion bodies was significantly reduced for arabinose-induced cultures compared to IPTG-induced cultures. The culture cell density was even increased since the supplemented arabinose could be used as an extra carbon source for cell growth. Most importantly, the specific PAC activity reached to a level higher than that of IPTG-induced cul-

Table 1 Comparison of IPTG and arabinose inducibility for *pac* expression in JM109(DE3) (pETKn29a) and BL21(DE3) (pETKn29a) under various induction conditions

JM109(DE3) (pETKn29a)									
Culture	1A1	1A2	1A3	1A4	1A5	1A6	1A7	1A8	1A9
IPTG (mM)	–[a]	0.05	0.1	0.2	0.5	–	–	–	–
Arabinose (g l^{-1})	–	–	–	–	–	1	5	10	15
Cell density (OD_{600})	2.2±0.1	1.5±0.1	1.1±0.1	1.0±0.1	1.0±0.1	2.6±0.1	2.5±0.1	2.5±0.1	2.5±0.1
Volumetric PAC activity (U l^{-1})	70±10	220±10	210±10	200±10	200±10	160±10	400±40	630±30	820±10
Specific PAC activity (U l^{-1} $OD_{600}{}^{-1}$)	34±4	160±10	200±10	200±10	210±10	62±5	150±10	250±10	330±10
BL21(DE3) (pETKn29a)									
Culture	1B1	1B2	1B3	1B4	1B5	1B6	1B7	1B8	1B9
IPTG (mM)	–	0.05	0.1	0.2	0.5	–	–	–	–
Arabinose (g l^{-1})	–	–	–	–	–	1	5	10	15
Cell density (OD_{600})	2.1±0.1	2.2±0.1	2.2±0.1	2.2±0.1	2.2±0.1	2.4±0.1	2.4±0.1	2.3±0.1	2.3±0.1
Volumetric PAC activity (U l^{-1})	8±2	120±15	150±10	160±10	170±10	18±8	240±10	270±30	290±40
Specific PAC activity (U l^{-1} $OD_{600}{}^{-1}$)	4±1	57±5	65±8	72±5	80±5	7±2	100±10	120±20	120±10

[a]The inducer was not used

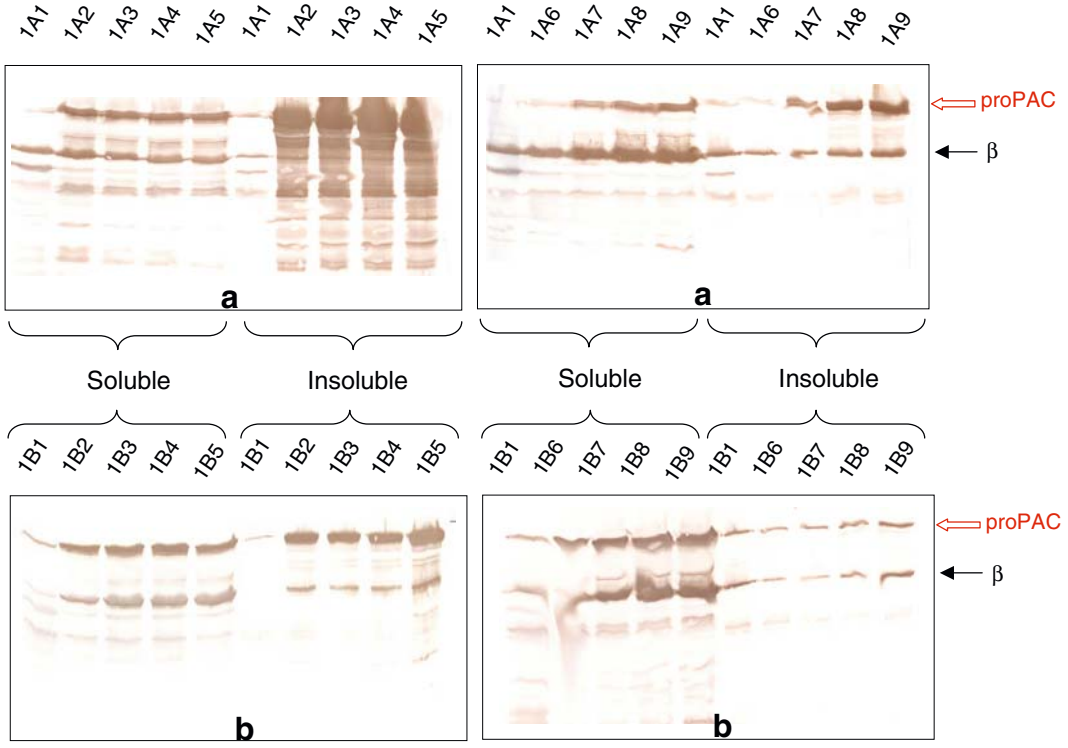

Fig. 1 Comparison of IPTG and arabinose inducibility for *pac* expression in JM109(DE3) (pETKn29a) (**a**) and BL21(DE3) (pETKn29a) (**b**): Western blotting analysis of soluble and insoluble protein fractions of culture samples from Table 1. Note that the soluble protein fractions on all Western blots (i.e., Figs. 1, 2, 3) amount to the intracellular fractions of the culture samples.

tures with an enhanced cell growth, resulting in a significantly improved volumetric PAC activity (e.g., 1A9 vs 1A5; 1B9 vs 1B5).

Effect of combined inducer and glycerol supplementation

The results of the simultaneous use of both IPTG and arabinose as inducers and the effect of glycerol supple-

Table 2 Effects of combined inducers (i.e., IPTG and arabinose) and glycerol supplementation on *pac* expression for JM109(DE3) (pETKn29a) and BL21(DE3) (pETKn29a)

JM109(DE3) (pETKn29a)							
Culture	2A1	2A2	2A3	2A4	2A5	2A6	2A7
IPTG (0.05 mM)	–[a]	+[b]	+	–	–	+	+
Arabinose (5 g l^{-1})	–	–	–	+	+	+	+
Glycerol (5 g l^{-1})	–	–	+	–	+	–	+
Cell density (OD_{600})	2.2±0.1	1.5±0.1	1.6±0.1	2.5±0.1	2.5±0.1	1.4±0.1	1.5±0.1
Volumetric PAC activity (U l^{-1})	70±10	220±10	460±40	400±40	380±10	620±30	690±20
Specific PAC activity (U l^{-1} $OD_{600}{}^{-1}$)	34±4	160±10	280±20	150±10	150±10	420±20	440±30
BL21(DE3) (pETKn29a)							
Culture	2B1	2B2	2B3	2B4	2B5	2B6	2B7
IPTG (0.1 mM)	–	+	+	–	–	+	+
Arabinose (10 g l^{-1})	–	–	–	+	+	+	+
Glycerol (5 g l^{-1})	–	–	+	–	+	–	+
Cell density (OD_{600})	2.1±0.1	2.2±0.1	2.2±0.0	2.3±0.1	2.3±0.1	2.2±0.1	2.2±0.1
Volumetric PAC activity (U l^{-1})	8±3	150±10	200±20	270±20	200±20	460±40	430±30
Specific PAC activity (U l^{-1} $OD_{600}{}^{-1}$)	4±2	65±8	85±10	120±10	87±7	210±20	200±20

[a]The supplement at the specified concentration was unused
[b]The supplement at the specified concentration was used

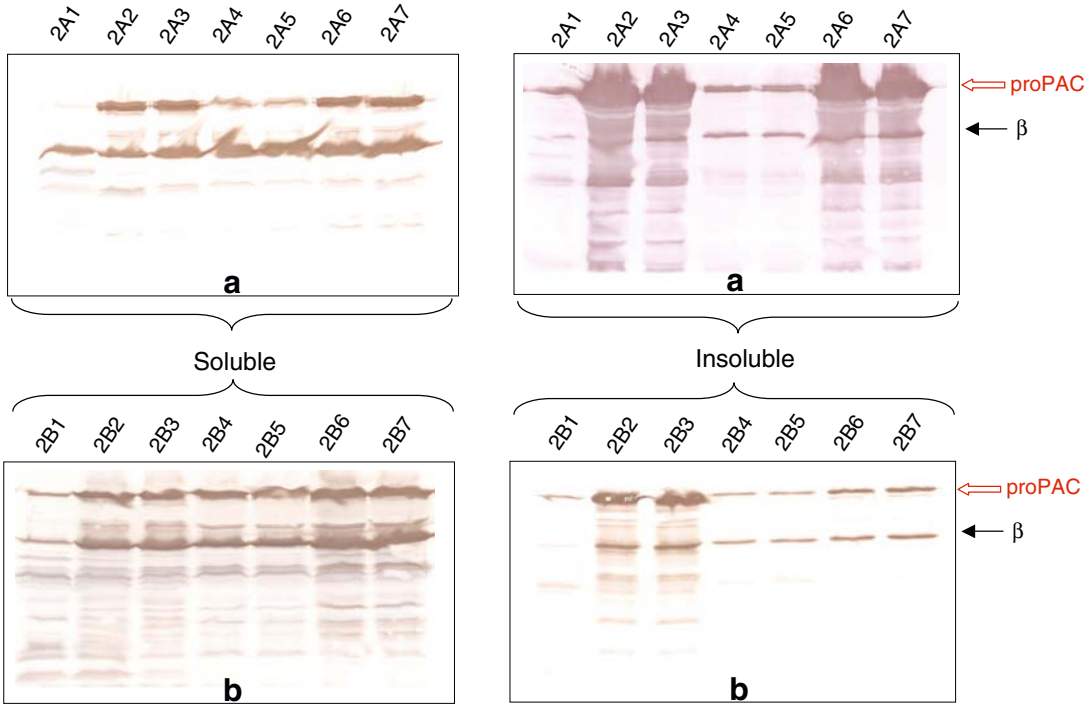

Fig. 2 Effects of combined inducers (i.e., IPTG and arabinose) and glycerol supplementation on *pac* expression for JM109(DE3) (pETKn29a) (**a**) and BL21(DE3) (pETKn29a) (**b**): Western blotting analysis of soluble and insoluble protein fractions of culture samples from Table 2

mentation are summarized in Table 2 and Fig. 2. For both hosts, while arabinose tended to suppress the formation of proPAC inclusion bodies mediated by IPTG induction, the induction effects from the two inducers on the formation of mature PAC were additive [i.e., (2A2 + 2A4) vs 2A6 and (2B2 + 2B4) vs 2B6]. Glycerol, although without inducibility on *pac* expression, appeared to have a similar effect in suppression of inclusion body formation, resulting in an improved PAC activity (i.e., 2A3 vs 2A2 and 2B3 vs 2B2). This improvement was particularly clear for JM109(DE3), in which the production of PAC was adversely affected by the overaccumulated proPAC inclusion bodies. However, neither arabinose nor glycerol could suppress the cell lysis caused by *pac* overexpression in JM109(DE3). Also, the above glycerol effect was not clearly observed when the culture was supplemented with arabinose, implying that

Table 3 Effects of combined inducers (i.e., IPTG and arabinose) and glycerol supplementation on *pac* expression for JM109(DE3) (pETKn29a, pARDegP) and BL21(DE3) (pETKn29a, pARDegP)

JM109(DE3) (pETKn29a, pARDegP)							
Culture	3A1	3A2	3A3	3A4	3A5	3A6	3A7
IPTG (0.05 mM)	–[a]	+[b]	+	–	–	+	+
Arabinose (5 g l^{-1})	–	–	–	+	+	+	+
Glycerol (5 g l^{-1})	–	–	+	–	+	–	+
Cell density (OD_{600})	2.1±0.1	1.7±0.1	2.0±0.1	2.4±0.1	2.3±0.1	2.3±0.1	2.2±0.1
Volumetric PAC activity (U l^{-1})	50±10	190±20	480±60	230±10	190±10	940±30	850±40
Specific PAC activity (U l^{-1} OD_{600}^{-1})	25±5	110±20	230±20	90±5	80±5	430±10	390±20
BL21(DE3) (pETKn29a, pARDegP)							
Culture	3B1	3B2	3B3	3B4	3B5	3B6	3B7
IPTG (0.1 mM)	–	+	+	–	–	+	+
Arabinose (10 g l^{-1})	–	–	–	+	+	+	+
Glycerol (5 g l^{-1})	–	–	+	–	+	–	+
Cell density (OD_{600})	2.3±0.1	2.3±0.1	2.6±0.1	2.3±0.1	2.5±0.1	2.3±0.1	2.3±0.1
Volumetric PAC activity (U l^{-1})	26±8	180±10	220±20	200±10	150±10	600±20	470±50
Specific PAC activity (U l^{-1} OD_{600}^{-1})	12±5	80±5	86±9	85±7	58±5	260±10	200±20

[a] The supplement at the specified concentration was unused
[b] The supplement at the specified concentration was used

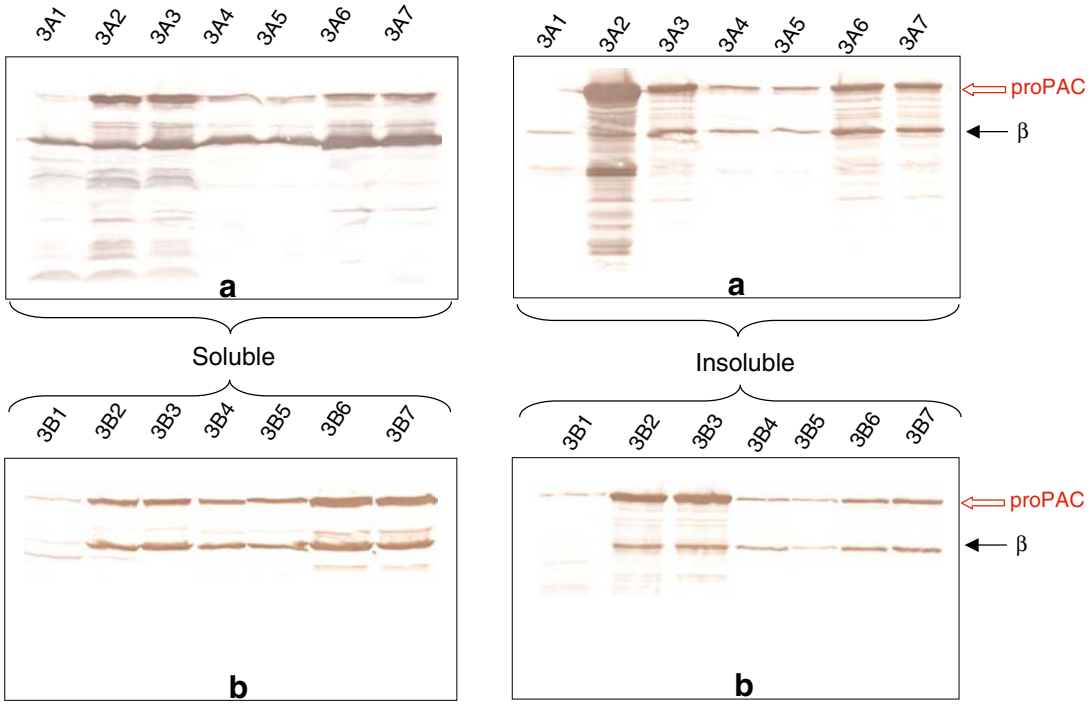

Fig. 3 Effects of combined inducers (i.e., IPTG and arabinose) and glycerol supplementation on *pac* expression for JM109(DE3) (pETKn29a, pARDegP) (**a**) and BL21(DE3) (pETKn29a, pARDegP) (**b**): Western blotting analysis of soluble and insoluble protein fractions of culture samples from Table 3

their role in reducing the amount of inclusion bodies was overlapping to some extent.

Effect of *degP* coexpression

The presence of exogenous DegP was shown to be able to enhance the production of PAC by suppression of various negative cellular responses to *pac* overexpression regulated by the *trc* promoter system (Lin et al. 2001; Pan et al. 2003). Such genetic strategy for PAC overproduction was further validated in the current T7 promoter system. Since P_{araB} is the most common arabinose-inducible promoter, one is interested in observing the inducibility for the gene expression system in which the two arabinose-inducible promoters [i.e., (DE3)/T7 and *araB*] coexist. Using JM109 (DE3) or BL21(DE3) harboring pETKn29a and pARDegP, *pac* expression performance is summarized in Table 3 and Fig. 3. However, upon adding IPTG only, *pac* expression was induced with inclusion body formation, particularly for recombinant JM109(DE3). Negative cellular responses of growth inhibition and cell lysis were also observed for recombinant JM109(DE3). The expression of *pac* for the binary plasmid system could be also induced by arabinose without negative cellular responses, even for recombinant JM109(DE3). Note that synergistic enhancement on the production of PAC was observed with simultaneous addition of IPTG and arabinose, including increased *pac* expression level, reduced inclusion body formation, no growth inhibition, and no cell lysis [e.g., 3A6 vs (3A2 + 3A4) or 3B6 vs (3B2 + 3B4)]. An extremely high *pac* expression level at 430 U l^{-1} $OD_{600}{}^{-1}$ was achieved for binary plasmid JM109(DE3) with simultaneous addition of IPTG and arabinose. The results suggest that the synergistic enhancement was primarily mediated by the presence of DegP and was observable only when IPTG was used as a primary inducer to induce *pac* overexpression.

Discussion

The formation of inclusion bodies, primarily composed of the insoluble gene product of proPAC, was previously identified as a major bottleneck limiting *pac* overexpression regulated by the *trc* promoter system (Pan et al. 2003; Xu et al. 2005). In addition, negative cellular responses such as inhibited cell growth and cell lysis were frequently observed upon *pac* overexpression (Pan et al. 2003). Most of these technical problems existed in the current T7 promoter system with IPTG induction for *pac* expression, particularly with JM109(DE3) as the expression host. While IPTG is known as the most common inducer, we have demonstrated that arabinose outperformed IPTG as another effective inducer for induction of the T7 promoter system for *pac* expression. The solubilization of proPAC was highly increased, as reflected by the reduced amount of inclusion bodies. The processing of proPAC became more efficient, as reflected by a less amount of accumulated soluble proPAC and the increased PAC activity. In addition, for recombinant JM109(DE3), the physiological burden caused by *pac* overexpression appeared to be diminished upon arabinose induction, as reflected by

enhanced cell growth and alleviated cell lysis, while maintaining a high *pac* expression level. All of the above positive effects translate into improved culture performance, as reflected by a significantly increased volumetric PAC activity.

The arabinose induction mechanism can be possibly mediated through the binding of arabinose or one of its derived metabolites to the *lac* repressor latching onto the *lac* operator, leading to the production of T7 RNA polymerase. However, the binding affinity of arabinose or arabinose-derived inducer (with the induction concentration in millimolar range) is rather weak compared to that of IPTG (with the induction concentration in micromolar range). Although there is no previous report regarding the binding of arabinose or its analog/derivative to *lac* repressor, the predicted secondary structure of the core domain of *lac* repressor exhibits excellent agreement with the known secondary structure of arabinose-binding protein (ABP) upon the primary sequence alignment (Nichols et al. 1993), indirectly implying the above-proposed binding possibility.

The formation of active PAC requires a series of unique posttranslational processing steps. Such processing is usually overwhelmed by the excess amount of transiently formed proPAC in either the periplasm (Chou et al. 1999) or the cytoplasm (Xu et al. 2005) upon *pac* overexpression regulated by the *trc* promoter. Apparently, the same technical problem existed in the current T7 promoter system, particularly with JM109(DE3) as the host. In contrast, more soluble proPAC appeared to occur in BL21(DE3). Since the overaccumulated proPAC that cannot be immediately processed for maturation tends to form inclusion bodies, it is believed that the production of PAC is limited not only by proPAC misfolding but also by inefficient proPAC processing. Strategies for enhancing the production of PAC should be targeted on improving both solubilization and processing of proPAC. While coexpression of chaperones (Xu et al. 2005) appears to be feasible for proPAC solubilization, which is a critical prerequisite for PAC maturation, the strategy, however, does not always guarantee the simultaneous improvement of proPAC processing. So far, no practical approach with a theoretical basis has been proposed for improving the intramolecular autoproteolysis of proPAC (Kasche et al. 1999), which critically limits the processing of proPAC for PAC maturation (Hewitt et al. 2000).

Apparently, arabinose was shown not only to induce *pac* expression regulated by the T7 promoter system but also to facilitate the folding and processing of proPAC, resulting in significantly improved culture performance for the production of PAC. Such facilitation of proPAC folding could be possibly mediated by the presence of arabinose as this is the only culture factor that did not exist in IPTG-induced cultures. There are reports indicating that sugar molecules are able to balance the chemical potential of overproduced proteins (Sawyer et al. 1994). Glycerol had an effect similar to, but not as significant as, arabinose in facilitating proPAC folding. It is noteworthy that glycerol has been known as a chemical chaperone for assisting protein folding in vitro (Futami et al. 2000; Sawano et al. 1992; Tsumoto et al. 2003) and in vivo (Figler et al. 2000; Lim et al. 2000, 2004). More studies at a molecular level are required for better understanding of the above sugar-mediated effects on the production of PAC.

The presence of exogenous DegP was previously shown to enhance the production of PAC by reducing the amount of periplasmic inclusion bodies and suppressing various negative cellular responses upon *pac* overexpression through IPTG induction of the *trc* promoter system (Pan et al. 2003). Such effect mediated by *degP* coexpression was also observable upon *pac* overexpression through IPTG induction of the current T7 promoter system, indicating that the similar technical problems (i.e., inefficient folding and processing of proPAC) arose. Note that the presence of DegP appears to both enhance proPAC solubilization and facilitate PAC maturation. On the other hand, the arabinose effects on the production of PAC identified in this study (i.e., increased PAC activity, reduced inclusion body formation, reduced cell lysis, and enhanced cell growth) are quite similar to those of *degP* coexpression, suggesting that the two approaches might share a similar mechanism for enhancing the production of PAC.

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