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Heterologous protein production in *Escherichia coli* using the propionate-inducible pPro system by conventional and auto-induction methods

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Abstract

We examined expression of two plant genes encoding coclaurine *N*-methyltransferase (CMT) and norcoclaurine synthase (NCS) in *Escherichia coli* from the *Salmonella enterica* *prpBCDE* promoter (P_{prpB}) and compared it to that from the strongest IPTG-inducible promoter, P_{T7} . In contrast to our previous study showing slightly higher production of green fluorescent protein (GFP) from the pPro system compared to that from the T7 system, production of two plant proteins CMT and NCS from P_{prpB} was 2–4 fold higher than that from P_{T7} . Unlike P_{T7} , expression from P_{prpB} did not reduce cell growth even when highly induced, indicating that this propionate-inducible system is more efficient for overproduction of proteins that result in growth inhibition. In an auto-induction experiment, which does not require monitoring the culture or adding inducer during cell growth, the pPro system exhibited much higher protein production than the T7 system. These results strongly indicate that the pPro system is well-suited for overproduction of recombinant proteins.

Keywords

auto-induction; *Escherichia coli*; gene expression system; pPro system; *prpBCDE* promoter; T7 promoter

Introduction

High-level, heterologous, protein production is very important for both basic research and practical applications. The phage T7 promoter (P_{T7}), known for its strength, has been commonly used for gene overexpression in *Escherichia coli* [1,2]. Upon induction by isopropyl β -D-thiogalactopyranoside (IPTG), the gene fused to P_{T7} can be highly expressed. However, it has several considerable drawbacks, including the high cost of IPTG and its toxicity to humans, which limits its use for production of human therapeutic proteins [3,4], and the reduction in cell growth when expression from P_{T7} is induced [5,6]. In addition, upon IPTG-

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induction of P_{T7}, formation of insoluble protein aggregates, known as inclusion bodies, is frequently observed [7]. Sometimes the host cells exhibit growth retardation resulting from the diversion of metabolism from all cellular processes to production of plasmid DNA, plasmid-encoded RNA, and plasmid-encoded proteins [8–10]. The physiological burden often results in growth inhibition, cell lysis, or even death [9,11]; thus, it is important to balance heterologous protein production with cell growth to maximize the overall protein production levels.

In general, it is difficult to choose an adequate promoter system and host for a specific protein, as it often depends on the nature of the target protein itself. Therefore, the ideal promoter for expression of recombinant proteins in *E. coli* must be inducible to varying degrees in single cells in order to minimize metabolic burden and protein toxic effects of the heterologous protein. Recently, we reported the regulation of the propionate-inducible *E. coli* *prpBCDE* promoter (P_{*prpB*}) [12] and developed two heterologous expression systems based on the *E. coli* and *Salmonella* P_{*prpB*} [13–16]. These systems showed regulatable expression at varying inducer levels, consistent expression in all cells of the culture, and strong expression at high inducer concentrations. In fact, the *S. enterica*-based expression system, consisting of P_{*prpB*} and the regulatory gene *prpR*, showed slightly higher GFP production than the T7 system. Finally, the induction is simple and cost-effective [14].

Although P_{*prpB*} appears to have a number of advantages over P_{T7} for production of the non-toxic and highly expressible GFP, further studies were necessary to examine its use for production of heterologous proteins. Additionally, we wanted to develop an auto-induction method similar to that developed by Studier [17] for producing proteins from the IPTG-inducible P_{T7} expression system. The auto-induction method is a convenient and efficient induction strategy that eliminates the need to monitor cell growth and add inducer at the appropriate time during growth to avoid growth inhibition. The auto-induction mechanism is based on diauxic growth resulting from dynamic function of *lac* operon regulatory elements in the auto-inducing medium containing glucose as the carbon source and catabolic repressor of P_{T7} during the cell growth stage. During the protein production stage, glycerol serves as the carbon source, and lactose as the inducer. Thus, heterologous gene expression is repressed during the cell growth stage and automatically induced by lactose after glucose exhaustion, resulting in little leaky expression during the initial cell growth stage, which in turn reduces cell growth inhibition.

In this study, we developed an auto-induction method for the propionate-inducible P_{*prpB*} system and examined its use for production of two plant proteins, the 44-kDa coclaurine *N*-methyltransferase (CMT) and the 25-kDa norcoclaurine synthase (NCS). We demonstrate that the use of the *Salmonella*-based expression system in *E. coli* increased protein production four fold over that using the T7 system, and in contrast to the T7 system, showed no growth inhibition. We believe that this system is highly complementary to the widely used T7 promoter expression systems for overproduction of toxic proteins.

Materials and methods

Bacterial strains and media

All DNA manipulations were performed in *E. coli* DH10B by using established protocols [18]. *E. coli* strain BLR(DE3) [BL21(DE3)/*recA*[−]] (Novagen) was the host for the pPro and T7 expression systems. Cells were grown in Luria-Bertani (LB) medium at 30°C. Cell growth was monitored as the optical density at a wavelength of 600 nm (OD₆₀₀). For auto-induction experiments [17], *E. coli* cells were grown in non-inducing medium (MDAG for agar plates or MDG for seed culture) and induced in the auto-induction medium (ZYM-5052). Media were supplemented with ampicillin (100 µg/mL) for pPro29b derivatives or kanamycin (50 µg/mL) for pET29b derivatives. IPTG, lactose, or sodium propionate (pH 8.0) was used as an inducer.

Plasmid construction

pPro18(S) was constructed by replacing the *araC* gene and the P_{BAD} promoter of pBAD18 [19] with the PCR-amplified *prpR* and P_{prpB} of *S. enterica*. To construct pPro29b(+), the PCR-amplified DNA fragments between XbaI and Bpu11021 restriction sites containing RBS, N-terminal S• Tag, and C-terminal His• Tag of the pET29b(+) vector (Novagen) using primers with NheI and SphI, respectively, were cloned into NheI and SphI digested pPro18(S). Two plant genes encoding coclaurine *N*-methyltransferase (AY217336) and norcoclaurine synthase (AY376412) were expressed as an N-terminal fusion with the S• Tag epitope (without C-terminal His• Tag) on pPro29b(+) or pET29b(+) vector, creating pPro-CMT and pPro-NCS, and pT7-CMT and pT7-NCS, respectively.

Protein expression

E. coli BLR(DE3) (Novagen) cells were transformed with the recombinant plasmids and grown on LB agar plates containing 100 µg/mL ampicillin or 50 µg/mL kanamycin for pPro29b(+) and pET29b(+) derivatives, respectively. Individual colonies were grown in culture tubes (25 mm x 150 mm) at 30°C at 200 rpm overnight in 3 mL LB medium supplemented with an antibiotic. Overnight grown cells were subcultured (1:100) into fresh 20 mL LB medium in 250-ml non-baffled shake flasks (Erlenmeyer) containing antibiotic. Cells were grown at 30°C at 200 rpm and induced with IPTG or propionate at the indicated OD values. For auto-induction [17], cells grown in non-inducing media were inoculated (1:100) into auto-inducing culture media supplemented with an antibiotic and inducer (propionate or lactose).

Protein assays

Cells from culture media were pelleted by centrifugation, resuspended in 100 mM Tris-HCl (pH 8.0) for soluble proteins or 8 M urea for total proteins or insoluble proteins, sonicated, and then centrifuged again. After centrifugation (20,000 x g at 4°C for 25 minutes), the supernatant was collected and stored at 4°C or -20°C. The Bradford protein assay method (Bradford reagent, Sigma-Aldrich) was used for general determination of protein concentrations. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Invitrogen), proteins were visualized by coomassie-blue staining (SimplyBlue™ SafeStain, Invitrogen), silver staining (SilverQuest™, Invitrogen), or Western blotting with S-protein alkaline phosphatase conjugate. For the latter detection scheme, the proteins on the polyacrylamide gel were transferred to PVDF membranes after SDS-PAGE with a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad). The electrophoretic transfer was conducted at a constant voltage of 125 V for 75 min. The blotting and detection were done using an amplified alkaline phosphatase immunoblot assay kit (Bio-Rad Laboratories, Hercules, CA). The FRETWorks™ S• Tag Assay Kit (Novagen) was used for assay of CMT and NCS S-tag fusion protein quantification according to the manufacturer's instruction. Fluorescence was measured in the Tecan SpectraFluor Plus plate reader (Tecan-US, Durham, N.C.) with an excitation wavelength of 492 nm and an emission wavelength of 530 nm.

Results

Effect of addition of IPTG or propionate into the culture media on cell growth

We investigated the effect of IPTG or propionate on cell growth by adding these compounds into culture media containing *E. coli* BLR(DE3) cells without any plasmids. The compounds were added when the culture optical density measured at a wavelength of 600 nm (OD_{600}) reached approximately 0.6; the OD_{600} was measured again after eight hours of cultivation. There were no changes in final OD_{600} between cells cultured with 0.5 mM IPTG and with 0 mM IPTG, indicating that IPTG itself is not toxic to *E. coli* host cells (Fig. 1). It has been reported that propionate is a poor carbon source for *E. coli*, and that *E. coli* requires a lag phase

of 4–7 days to adjust to growth on propionate as the sole carbon source [20]. Interestingly, addition of 5 mM or 15 mM propionate increased the final cell mass by 10 or 20%, respectively, compared with cells cultured in the absence of propionate.

Time course of cell growth and gene expression

Cell growth and protein production from the T7 and the pPro expression systems were determined at various times following induction. The levels of the 44-kDa CMT in the soluble fraction saturated after 8 h of induction in both systems. At 8 h of cultivation in LB medium containing 20 mM propionate, a dramatic increase in OD₆₀₀ was observed in cultures harboring pPro-CMT over cultures without any plasmid (Fig. 1 and 2). This may be the result of increases in cell size due to overproduction of expressed proteins [5,21], subsequently resulting in the increased OD₆₀₀. However, cultures expressing CMT from the T7 system showed much lower OD₆₀₀ than cultures expressing CMT from the pPro system after 8 h of induction, indicating that there was a serious decrease in the growth rate of cells harboring pT7-CMT relative to those harboring pPro-CMT.

The T7 system showed a slightly faster rate of induction, probably resulting from superior processivity of T7 RNA polymerase [1]. Compared with the T7 system, the pPro system had a time delay before high protein production (Fig 2), indicating that this system requires time to express the transcriptional activator PrpR and the enzymes PrpC and PrpE, which produce the inducer, 2-methylcitrate (2-MC), from exogenous propionate [12,13].

Comparison of protein production and cell growth from pPro and T7 expression system at various inducer concentrations

Two plant genes (encoding the 44-kDa CMT and 25-kDa NCS) were expressed under the control of P_{prpB} or P_{T7} in *E. coli* BLR(DE3). The protein expression levels and cell growth were measured at 8 h post-induction as a function of the inducer concentration (Fig. 3). Consistent with previous studies done with the gene encoding the green fluorescent protein (*gfp*) [13–16], the *Salmonella*-based pPro system was regulatable over a wide range of inducer concentrations, and protein production levels depended on the extracellular propionate concentration. In contrast, the T7 system showed almost no dose-dependent IPTG inducibility.

The T7 system induced with high concentrations of IPTG exhibited a significant decrease in cell growth. Specifically, the most serious growth inhibition was observed in cultures carrying pT7-NCS, indicating that the effects of recombinant protein production on growth rate were protein specific. Overexpression of NCS from the strong P_{T7} in the fully induced cells may result in stopping cell growth even at the lower concentrations of IPTG. However, the pPro system did not inhibit cell growth even at the highest propionate concentrations. Cultures harboring pT7-CMT and induced with 0 to 50 μM IPTG showed a slight increase in OD₆₀₀ as well as protein production (Fig. 3A and B), probably due to an increase in cell size as a consequence of the highly expressed proteins [5,21].

Effect of the density of the culture on protein production

The inhibition of bacterial growth upon induction of pT7-CMT or pT7-NCS resulted in a decreased amount of both biomass and recombinant proteins when induced with relatively high concentrations of IPTG. Thus, we tested the effect of inducing expression of NCS at high cell densities (OD₆₀₀ of 1.5 or 3.2). The T7 system inhibited growth even when induced at relatively high cell densities, and the degree of cell growth inhibition was dependent on IPTG concentration in the culture medium. This finding suggests that the response to foreign toxic protein overproduction is rapid and severe in the T7 expression system (Fig. 4). In contrast, the pPro system exhibited increased cell growth instead of growth inhibition even when induced with a high concentration of propionate (20 mM), possibly due to the combination of

the increase in cell size resulting from highly expressed protein and a small increase in cell growth as a result of utilization of propionate. Although the T7 system showed a small increase in OD₆₀₀ when induced at a relatively high OD, volumetric protein production did not increase significantly.

Comparison of protein production levels from the pPro and T7 expression systems

The CMT or NCS levels in the soluble and insoluble protein fractions were quantified by an assay method based on the interaction between the 15-amino acid S• Tag peptide with ribonuclease S-protein (Novagen) (Table 1). The pPro system produced higher amounts of the two proteins in both total protein and soluble protein fractions than the T7 system. The amounts of soluble CMT and NCS from the pPro system were 2.5- and 4-fold more than those from T7 expression system, respectively. This was the result of the increased final cell mass and higher specific expression (per cell mass) in the pPro system. The ratios of soluble to insoluble CMT were 1 and 0.5 in pPro and T7 systems, respectively, while the soluble/insoluble ratios of NCS were 4 in both expression systems. This supports the idea that the cell growth inhibition observed in cells expressing NCS from P_{T7} was not due to the formation of insoluble protein.

Comparison of protein production levels from the pPro and T7 expression systems in auto-induction media

To eliminate any problems caused by leaky expression from the T7 system and to ease induction of the pPro system, we compared NCS production levels between the T7 and pPro systems in auto-inducing medium after seed culturing in non-inducing medium [17]. Studier reported that auto-induction allows cell growth to high densities without leaky expression and efficient induction at high OD. In our experiments, the pPro system exhibited higher protein expression and OD₆₀₀ than the T7 system, even under auto-induction conditions (Fig. 5). Unlike pPro expression strains (lanes 3–10), the T7 expression strains (lanes 1–2) did not show increased OD₆₀₀ compared to uninduced cells (lanes 11 and 12 for T7 system and lanes 13 and 14 for pPro system), suggesting that lactose may not be metabolized for a carbon source or that fully induced T7 expression cells stop growing. The pPro system (lanes 3–6) worked well in auto-inducing media as expected, because it is subject to catabolite repression by glucose [12–14]. Moreover, the pPro system showed much less leaky expression than the T7 system, even in the presence of glucose (lanes 11–14). Also, there was no significant difference in NCS production from the pPro system between auto-induction (lanes 3–6) and normal induction (lanes 7–10) when the inducer was added when the OD₆₀₀ reached 3.5.

Discussion

Previously, we showed that the *Salmonella*-based pPro system was capable of slight high-level *gfp* expression compared to the IPTG-inducible T7 system [14,15]. Here, we compared overexpression of plant genes from the pPro system to that from the T7 system through auto-induction and conventional induction methods in terms of cell growth and soluble protein productivity, and demonstrated 2–4 fold higher protein expression of the pPro system over the T7 system. The ability of the pPro system to finely-regulate the expression levels is important for maximizing the production of recombinant toxic proteins. By properly tuning expression to balance growth with specific protein production, it is possible to maximize total protein production.

Typically, attempts to produce large quantities of a single protein in *E. coli* begin by placing a gene for the desired protein under the control of a very strong promoter such as the T7 promoter. However, it has been found that fully induced cells sometimes behave undesirably: they grow slowly, eventually stopping growth altogether, and produce insoluble protein [22–25]. There are several reasons why normal growth functions are disrupted by the production

of large quantities of a nonessential (non-functional, non-toxic) protein. First, given a fixed quantity of total protein in the cell, the overproduction of nonessential protein leads to a proportional reduction in the fraction of essential proteins [9,11]. Second, at the highest induction levels of heterologous protein, the bacteria degrade rRNA as well as previously assembled, intact ribosomes, with a concomitant crash of the translation system [9,11]. Finally, recombinant protein overexpression results in a rapid stress response such as increased protease activity [26], cell lysis [27], and up-regulation of heat shock, SOS/DNA damage and stationary phase genes [28]. The T7 system has additional disadvantages for protein overexpression: it is not a controllable system at the single cell level because most of the IPTG is transported into cells by active LacY transporter [29]. Thus, the T7 system suffers from all-or-none or autocatalytic gene expression. Upon induction with IPTG, there will be two populations (fully induced and un-induced cells) at low to intermediate inducer concentrations, the fraction of each depending on the inducer concentration. This fact makes it impossible to reduce cell stress by lowering toxic protein expression in induced cells. Also, the T7 RNA polymerase has the ability to transcribe up to eight times more rapidly than *E. coli* RNA polymerase [1,30]. Therefore, the population of fully induced cells produces a large amount of the heterologous protein in a short time, probably causing cell growth inhibition. In contrast, the pPro system is a regulatable system in single cells and uses the native *E. coli* RNA polymerase for transcription from P_{prpB} . In addition, the pPro system may have lower leaky expression during log-phase growth, because *prpBCDE* gene expression is known to be positively controlled by the stationary-phase-inducible sigma factor RpoS [31] and the integration host factor (IHF) involved in RpoS-dependent gene activation [32]. The combination of these advantages results in little or no significant cell growth inhibition and high protein production.

T7 RNA polymerase is so active that a small basal level can lead to substantial expression of the target protein, due to the presence of lactose in LB. If the target protein is toxic to the host cells, growth of seed cultures in LB can stress cells by unintended induction, enriching for cells that are less inducible and therefore reducing the yield upon IPTG induction of a subculture. Additionally, with long induction times, fully induced cultures can become overgrown with cells that are not competent for induction [17]. To solve these problems, Studier formulated a non-inducing medium for seed culture and an auto-inducing medium for auto-induction. The auto-induction increased the yield of toxic protein several fold over the previous IPTG induction conditions [17]. This increase may be mainly due to an increase in cell mass. For example, the OD₆₀₀ in auto-inducing culture was approximately 10-fold higher than in a culture induced with 0.5 mM IPTG. Although NCS is not very toxic to the cell, it is interesting that the pPro system produced more NCS than the T7 system in an auto-inducing medium. There are two possible explanations: one is that the heterologous protein production in a short time still affects cell growth and metabolism in the medium, and the other is that lactose is an inferior to IPTG as an inducer [33].

The addition of 15 mM propionate resulted in 20% increase in the final OD of *E. coli* cells without any plasmids (Fig. 1). One possibility for our observation of improved growth is that propionate can be co-metabolized in the presence of other preferred carbon sources, which exist in the LB medium. Another possibility is that propionyl-CoA produced from propionate improves cell growth by being metabolized via several possible propionate oxidation pathways, such as α -oxidation, β -oxidation, α -carboxylation, reductive carboxylation, and Claisen condensation [20]. Those possibilities do not explain the 1.7-fold higher OD from cells induced with 15 mM propionate compared to uninduced cells (Fig. 3 A4). The fact that the stationary-phase-inducible sigma factor RpoS is a positive effector for the pPro system [31] might cause lower background expression during log-phase growth, resulting in no cell growth inhibition and higher heterologous gene expression during stationary-phase, resulting in high protein production. Subsequently, the overproduction of the heterologous proteins may increase cell size, causing the increased OD₆₀₀ [5,21]. These properties of the pPro system may make it a

good expression system for the overproduction of heterologous proteins that result in growth inhibition.

The *Salmonella*-based pPro system is not without problems. The first is that the system is not independent of the host strain background. Thus the system is inactive in *E. coli* DH10B and 5 α , which lack the *prp* operon, as the real inducer 2-MC is made from propionate via propionyl-coenzyme A by using the chromosomally expressed *prpCE* gene products [13]. The second is that the system is not independent of the host strain metabolism like arabinose-inducible and lactose-inducible promoter expression systems. It is subject to catabolite repression by glucose or glycerol in LB medium but not in minimal medium [15]. Recently, we also found that the *Salmonella* pPro system was down-regulated by the presence of arabinose (unpublished data). These results are offering new perspectives for further studies to understand its performance among different culture conditions.

In summary, the *Salmonella*-based propionate-inducible expression system, consisting of the *prpBCDE* promoter (P_{prpB}) and the regulatory gene *prpR* from *Salmonella enterica*, is an excellent expression system for heterologous protein production in *E. coli*. Additionally, the system requires propionate for induction, which unlike IPTG is neither expensive nor toxic. Thus, this system is an attractive option for industrial and pharmaceutical protein production through the use of conventional induction or auto-induction method.

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Fig. 1.

Effect of the addition of IPTG or propionate on cell growth. *E. coli* BLR(DE3) cells without any plasmids growing at 30°C in LB were exposed to the indicated concentrations of inducer when the OD₆₀₀ reached 0.6 and incubated at 30°C for 8 h. IPTG (50 μM, or 500 μM) or propionate (5 mM, or 15 mM) was added into cultures when the OD₆₀₀ reached 0.6. Eight hours after addition, the OD₆₀₀ was determined. Prop, propionate. The data are representative of three independent experiments.

Fig. 2.

Comparison of time course induction for the expression of NCS between *E. coli* BLR(DE3) harboring pPro-CMT or pT7-CMT. Cultures of *E. coli* harboring pPro-CMT or pT7-CMT were induced with 0.5 mM IPTG or 20 mM propionate when the OD₆₀₀ reached 0.6 and incubated at 30°C. Aliquots were collected at the indicated intervals. Total soluble protein fraction was prepared as described in Materials and Methods. Soluble proteins (25 µg per lane) were separated on SDS/PAGE. Proteins were determined using Western blotting. The relative positions of protein markers are indicated on the right in kDa. CMT; coclaurine *N*-methyltransferase.

Fig. 3.

Comparison of protein expression levels with respect to the concentration of inducer. Cells growing at 30°C in LB were exposed to the indicated concentrations of inducer when the OD₆₀₀ reached 0.6 and incubated at 30°C for 8 h. (A) Soluble proteins (25 μg per lane) from BLR(DE3) cells harboring pT7-CMT or pPro-CMT were separated by SDS-PAGE and stained with Coomassie blue (A1) or subjected to Western blot detection (A2). Arrowhead on the left shows the position of expressed CMT, whereas those on the right point to the positions of molecular size markers. Expression was induced with the concentrations of IPTG (μM) or propionate (mM) indicated (A3). Cultures reached the final OD₆₀₀ indicated (A4). (B) Same as A, except that this experiment was performed with BLR(DE3) cells harboring pT7-NCS or pPro-NCS. CMT, coclaurine *N*-methyltransferase; NCS, norcoclaurine synthase.

Fig. 4.

Effect of the culture density of the culture on gene expression. Cells growing at 30°C in LB were exposed to the indicated concentrations of inducer at the indicated OD₆₀₀ values and incubated at 30°C for 8 h. (A) Cell mass (OD₆₀₀) of *E. coli* BLR(DE3) harboring pPro-NCS or pT7-NCS. Cultures were induced with 3 (white bars), 10 (gray bars) or 20 mM propionate (black bars), or 25 (white bars), 100 (gray bars) or 500 μM IPTG (black bars) at different OD₆₀₀ values (0.6, 1.5, 3.2) indicated. (B) SDS-PAGE analysis of total protein fraction. (C) SDS-PAGE analysis of soluble protein fraction. Total protein fraction and soluble protein fraction were collected from *E. coli* cells harboring pPro-NCS (lanes 1 through 6) or pT7-NCS (lanes 7 through 12) after induction of 8 h with 10 (lanes 1, 3, and 5), 20 mM propionate (lanes 2, 4, and 6), or 25 (lanes 7, 9, and 11), 100 μM IPTG (lanes 8, 10, and 12) when OD₆₀₀ reached 0.6 (lanes 1, 2, 7, and 8), 1.5 (lanes 3, 4, 9, and 10) or 3.2 (lanes 5, 6, 11, and 12). Protein samples corresponding to 70 μl of culture were analyzed by SDS-PAGE and silver staining.

Fig. 5.

Comparison of NCS production between *E. coli* BLR(DE3) cells harboring pT7-NCS (lanes 1, 2, 11, and 12) or pPro-NCS (lanes 3–10, 13, and 14) in ZYM-505 culture medium. *E. coli* cells grown on MDAG plates were inoculated into 3 mL of MDAG liquid media, cultivated overnight at 30°C, and then subcultured (1:100) into ZYM-505 supplemented with 0.2% lactose (lanes 1 and 2) or with 20 mM (lanes 3 and 4) or 30 mM (lanes 5 and 6) propionate for auto-induction, or into ZYM-505 (lanes 7–14) without inducer. *E. coli* BLR(DE3) cells (lanes 7–10) were induced with 20 mM (lanes 7 and 8) or 30 mM (lanes 9 and 10) propionate when OD₆₀₀ reached 3.5. Cells having pT7-NCS (lanes 11 and 12) or pPro-NCS (lanes 13 and 14) were used as controls and cultivated in ZYM-505 without addition of inducers. After 15 h (odd number lanes) or 18 h (even number lanes) cultivation in 20 mL culture medium in 250-mL flasks at 30°C with shaking (200 rpm), the soluble protein fraction was collected from the cell pellet. Soluble proteins corresponding to 6.25 µL of culture were analyzed by SDS-PAGE and silver staining. In lanes 11–14, a single sample was analyzed. Arrowhead on the right shows the position of expressed NCS.

Table 1
Comparison of quantitative protein production from the T7 and pPro systems.

Promoter Plasmid Gene expressed	<i>Salmonella</i> P _{ppB}			pPro-NCS		pT7-CMT		phage P _{T7}		pT7-NCS	
	pPro-CMT			norococlaurine synthase		coclaurine N-methyltransferase		coclaurine N-methyltransferase		norococlaurine synthase	
Inducer Conc. (Prop/IPTG)	15 mM	10 mM	5.3	15 mM	10 mM	50 μM	25 μM	50 μM	25 μM	50 μM	25 μM
Cell density (OD ₆₀₀)	5.5	41.2 ± 2.0	5.3	4.1	3.7	4.4	4.0	2.3	2.9	2.3	2.9
Soluble protein (nmol/mL)	47.0 ± 2.9	46.9 ± 3.4	41.2 ± 2.0	61.2 ± 7.2	39.2 ± 5.7	20.5 ± 1.2	13.3 ± 2.2	10.5 ± 1.0	15.3 ± 4.3	10.5 ± 1.0	15.3 ± 4.3
Insoluble protein (nmol/mL)	47.0 ± 1.0	46.9 ± 3.4	46.9 ± 3.4	18.2 ± 3.6	7.9 ± 0.8	53.7 ± 10.0	25.2 ± 2.0	2.2 ± 0.7	4.1 ± 2.0	2.2 ± 0.7	4.1 ± 2.0

Cells growing at 30°C in LB were exposed to the indicated concentrations of inducer when the OD₆₀₀ reached 0.6 and incubated at 30°C for 8 h. The abbreviations are as follows: (Prop) propionate, (Conc) concentration; (nmol/mL) means that amount of expressed proteins per 1 mL of culture medium; All results are expressed as means ± standard deviations from three independent experiments. Standard deviations of OD₆₀₀ were below 0.01 and hence are not shown.