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Optimization and High-level Expression of a Functional GST-tagged rHLT-B in *Escherichia coli* and GM1 Binding Ability of Purified rHLT-B

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The *Escherichia coli* heat-labile enterotoxin B subunit (HLT-B) is one of the most powerful mucosal immunogens and known mucosal adjuvants. However, the induction of high levels of HLT-B expression in *E. coli* has proven a difficult proposition. Therefore, in this study, the HLT-B gene was cloned from pathogenic *E. coli* and expressed as a fusion protein with GST (glutathion S-transferase) in *E. coli* BL21 (DE3), in an attempt to harvest a large quantity of soluble HLT-B. The culture conditions, including the culture media used, temperature, pH and the presence of lactose as an inducer, were all optimized in order to obtain an increase in the expression of soluble GST-rHLT-B. The biological activity of the purified rHLT-B was assayed in a series of GM1-ELISA experiments. The findings of these trials indicated that the yield of soluble recombinant GST-rHLT-B could be increased by up to 3-fold, as compared with that seen prior to the optimization, and that lactose was a more efficient alternative inducer than IPTG. The production of rHLT-B, at 92% purity, reached an optimal level of 96 mg/l in a 3.7 L fermentor. The specific GM1 binding ability of the purified rHLT-B was determined to be almost identical to that of standard CTB.

Keywords: expression optimization, lactose induction, rHLT-B purification, GM1 binding ability

The *E. coli* heat-labile enterotoxin B subunit (HLT-B) is one of the most powerful known mucosal immunogens and mucosal adjuvants, as it proven to be capable of eliciting a profound immunological response to co-administered antigens (Millar *et al.*, 2001; Richards *et al.*, 2001). Therefore, a great deal of effort has been focused on the preparation and application of HLT-B in large quantities (Hashiguchi *et al.*, 1996; Tamura *et al.*, 1997). However, few researchers have actually succeeded in producing rHLT-B in *E. coli* with sufficiently high yields. This may be attributable to the fact that HLT-B is a multimeric protein, belonging to the AB5 type entertoxin family. The structural complexity, heat-lability, low solubility, and generally low stability of this protein are all factors which would tend to hamper the efficient expression of the protein in *E. coli* (Nashar *et al.*, 1996). Therefore, some studies have focused on the production of HLT-B via the use of other eukaryotic expression systems, including yeast and transgenic tobacco systems (Birgit *et al.*, 2004; Mohammad *et al.*, 2005), both of which tend to be inconvenient or low-yielding as compared to *E. coli* systems. In this study, we have attempted to utilize *E. coli* expressing HLT-B at high levels in *E. coli,* using a soluble fusion-tagged GST and a lactose inducer.

As has already been firmly established, *E. coli* is one of the most convenient and most extensively used hosts for the expression of recombinant proteins. However, a variety of proteins have proven to be quite difficult to express in an active form using *E. coli* systems. As compared to the intense effort inherent to the dissolution of inclusion bodies and the refolding of recombinant proteins, it appears to be necessary, from an economic perspective, to explore alternative methods for the maximization of the yield of the expressed protein, in both its soluble and active forms (Hellman *et al.*, 1995). Several methods might be used to enhance the expression the heterogenous protein. The co-expression of molecular chaperones has been proven to exert a positive effect on the expression of recombinant proteins (Thomas and Baneyx, 1996), although the extent to which this improvement is observed depends principally on the

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nature of the protein. Increases in the cultivation temperature tend to enhance production, but high temperature, in some cases, can generate negative effects on both solubility and stability. Therefore, optimal fermentation parameters need to be established for the expression of different proteins.

Findings with regard to the HLT action mechanism indicate that B subunits are responsible for target cell recognition, and possess a high degree of affinity with the natural receptor-binding site, ganglioside GM_1, which harbors lactose as its terminal sugar (Sixma *et al.*, 1992). Therefore, the yield of rHLT–B might be improved via the supplementation of the culture medium with lactose, which can function as a nutrient and building block of this protein. Lactose is a common agent, and is widely used in laboratories. Lactose, an analogue of IPTG, is also capable of inducing *lac* and its derivative promoters for the expression of target recombinant proteins, after the lactose has been transformed into allolactose by *E. coli* (Donovan *et al.*, 1996; Yildirim and Mackey, 2003). Its low cost and lack of toxicity make lactose an appropriate inducer for both vaccine and drug engineering applications. The optimal fermentation parameters tend to vary greatly in cases in which lactose is employed as an inducer for the expression of recombinant proteins. It is, then, necessary to determine these optimal parameters for individual cases, via the performance of a large number of optimization experiments (Menzella *et al.*, 2003).

In this study, we describe the soluble expression of rHLT-B, using GST as a soluble fusion-tag in *E. coli* BL21 (DE3) under optimized cultural conditions, using lactose, rather than IPTG, as the inducer. The paper also include a description of the purification process, as well as the characterization of the biological activity of rHLT-B. Other related procedures, including gene cloning and recombinant plasmid construction, are also described.

Materials and Methods

Bacterial strains, plasmids, enzymes, reagents and media

A pathogenic strain of *E. coli,* O6:H16 (LT^+, ST^+), which was generously provided by Dr. Zhijiang Zhou (University of Tianjin, China), was used for the cloning of the HLT-B gene. *E. coli* DH5α and BL21 (DE3) were obtained from Novagen (Germany). The pGEM-T and pGEX-4T-1 plasmids were acquired from Promega and Pharmacia Biotech, respectively. The restriction enzymes, T4 DNA ligase, RNase, Thrombin, Pyrobest™ DNA polymerase, and DNA Marker were purchased from Takara. The Pfu/A Amp Tailing Kits used in this study were obtained from Shanhai Sangon of China. Standard CTB was purchased from Sigma. Lactose, IPTG and other chemicals used in this study were all of analytical or higher grades. Goat anti-CT-B polyclonal antibody, which was used as the primary antibody, and rabbit anti-goat HRP-IgGFc conjugate, which was used as the secondary antibody, were purchased from Santa Cruz Biotechnology. The LB (tryptone 10 g/l, yeast extract 5 g/l, NaCl 10 g/l), 2 × YT (tryptone 16 g/l, yeast extract 10 g/l, NaCl 5 g/l) and TB (tryptone 12 g/l, yeast extract 24 g/l, glycerol 4 ml/l, KH_2PO_4 2.31 g/l, K_2HPO_4 12.54 g/l) culture media were prepared as described elsewhere.

Cloning and sequencing of the HLT-B gene and construction of the expression vector

The bacterial plasmids from the *E. coli* strain O6:H16 (LT^+, ST^+) were prepared in accordance with a method described elsewhere (Sambrook and Russel, 2001). PCR amplifications were conducted in 50 μl reaction mixtures, each containing 1 μl plasmids from *E. coli* O6:H16 (LT^+, ST^+) as a template, 2.5 units of Pyrobest™ DNA polymerase, 5 μl PCR 10 × buffer, 25 mM each dNTP, and 25 pmol each of Pb1 and Pb2 primer designed according to HLT-B mRNA (GenBank accession AF242418). The PCR conditions were as follows: an initial denaturation for 5 min at 96°C, followed by 30 cycles of denaturation at 96°C for 30 s, annealing for 30 s at 55°C, extension at 72°C for 30 s, and a final extension for 7 minutes at 72°C. The following primers were used: primer Pb1: 5'-GAATTCGCTCCCCAGACTATTACAGAA-3' (forward: 64-85), harboring an introduced *Eco*R I recognition sequence; and primer Pb2: 5'-GCGGCCGC CTAGTTTTCCATACTGATTGCCGC-3' (reverse: 351 -375), harboring an introduced *Not*I recognition sequence. The amplified PCR products, after clean-up and A-tailing reaction were performed using the Pfu/A Amp Tailing Kit, were cloned into the pGEM-T vector. The recombinant plasmid was then transformed into DH5α competent cells. The transformed cells were cultured on LB/Amp plates, with IPTG/X-gal.

Recombinant white bacterial clones were selected. The HLT-B gene was subcloned into pGEX-4T-1, using *Eco*RI and *Not*I, resulting in the expression of the recombinant plasmid. Standard recombinant techniques were employed in the construction of the pGEX-4T-1-rHLT-B vector (Fig. 1). The recombinant vector harboring the HLT-B gene was verified via restriction endonuclease digestion and sequencing.

Expression of the HLT-B gene in E. coli and cell disruption

An *E. coli* BL21 (DE3) clone harboring the pGEX-4T-1-rHLT-B plasmid was grown in LB medium supplemented with 50 μg/ml ampicillin. 100 milliliters

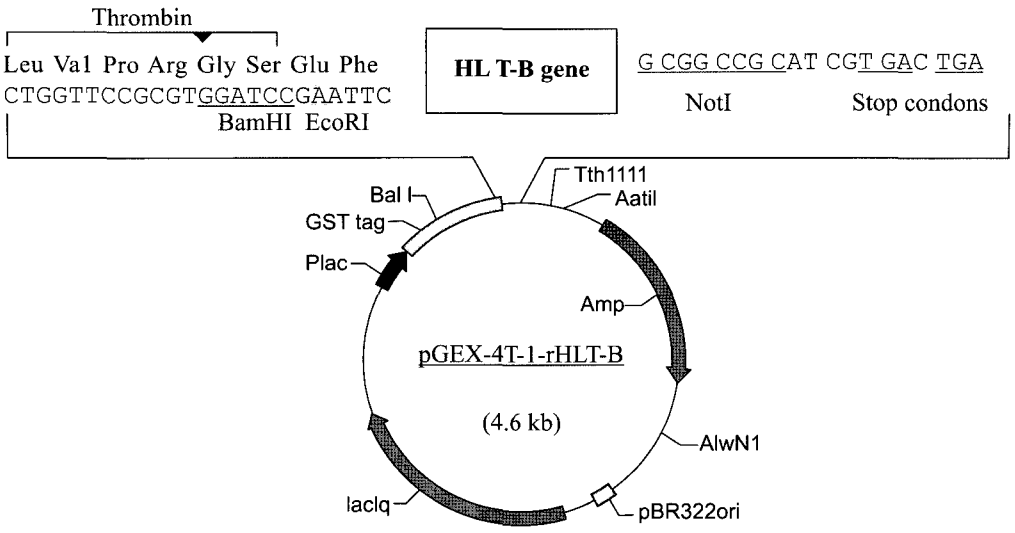

Fig. 1. Schematic representation of the expression vector pGEX-4T-1-rHLT-B. The *tac* promoter, fusion partner of GST, *lacI*^{q} gene, thrombin cleavage site and ampicillin resistance are indicated.

of fermentation medium containing 50 μg/ml ampicillin was inoculated with culture, to an optical density of 0.6 ($O.D._{600}$) at a ratio of 5% (v/v), for the generation of the recombinant protein. The bacteria were allowed to grow at 37°C in a shaking incubator at 250 rpm. The protein was expressed via lactose induction, to a concentration of 10 g/l. After an additional 5 hours of cultivation, the bacteria were harvested via 10 minutes of centrifugation at 6,000 × *g*. The cells were re-suspended in 20 mM Tris-HCl (pH 7.5) and lysed via sonication. The soluble proteins in the supernatant were harvested after an additional 10 minutes of centrifugation at 12,000 × *g*. The precipitate was discarded.

Effects of different media and pH on GST-rHLT-B expression

Cell growth profiles in three kinds of media, LB, 2 × YT and TB, were evaluated. A preliminary comparison of protein yields in the three media was also conducted, via SDS-PAGE. The best of these media was then employed for subsequent experiments. The effects of different pH values (6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) were determined via comparisons of the yields of the protein of interest, according to the results of SDS-PAGE. The optimized pH value was maintained throughout the optimization of the remaining cultivation parameters.

Effects of different inducing temperatures and time on GST-rHLT-B expression

The fermentation of the recombinant strain in the TB medium occurred in two distinct phases, namely the cell growth and foreign protein expression phases. The cells were permitted to grow for 12 hours at 37°C. After the addition of inducer, the bacteria were cultured at four different temperatures, 25°C, 30°C, 37°C, and 42°C. At each temperature, the induction period lasted for 5 h. The broth used in each of the treatments was sampled at identical induction times for different analyses.

Effect of inducing concentrations of lactose rather than IPTG as an inducer

Under optimized cultivation conditions, lactose was added to cultures, at final concentrations of 5, 10, and 50 g/l, at the same bacterial growth stages (A_{600}=0.6). The cultures were then continuously incubated with shaking at 250 rpm under the optimal inducing temperature and time conditions established above. IPTG with 0.5 and 1.0 mM was simultaneously used as an inducer control, the inducing effect of which on the expression of recombinant proteins had been confirmed in our previous studies. The expression and yield of GST-rHLT-B were determined and estimated via SDS-PAGE and using a Bio-Rad gel image analysis system.

Batch fermentation in a 3.7 L bench-top fermentor

E. coli BL21 bacteria (DE3) harboring the pGEX-4T-1-rHLT-B plasmid were cultured in TB medium at 37°C and a pH of 7.5 in a 3.7 L fermentor (Bioengineering, Sweden). Recombinant protein expression was induced using 10 g/l lactose at 25°C (pH 8.0) at an $O.D._{600}$ of 1.0. After 15 hours of induction, the cells were harvested via centrifugation, for further use in the purification procedure.

Purification of rHLT-B

Cells grown at 37°C (pH 8.0) and induced with lactose at a final concentration of 10 g/l for 5 h at 25°C in a 3.7 L fermentor, were then harvested via 15 minutes of centrifugation (12,000 × *g*) at 4°C. The supernatants were discarded. The cells were frozen at –20°C or immediately processed. 50 μl of ice-cold PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2PO_4, and 1.8 mM KH_2PO_4, 1% (v/v) Triton X-100, pH 7.3) per mL was added to the cell cultures, after which the cultures were sonicated at 400 W for 10 minutes at 4°C. The lysates were gently shaken for 30 minutes at room temperature in order to solubilize the fusion proteins, followed by 15 minutes of centrifugation at 12,000 × *g* at 4°C. The protease inhibitor, PMSF (phenylmethylsulfonylfluoride) was added to the supernatants, to a final concentration of 0.1 mg/ml. Two milliliters of Glutathione-Sepharose 4B resin, which had been previously equilibrated in PBS, were added to 100 ml of the supernatants from the sonication step. This mixture was subjected to gentle agitation at room temperature, in order to allow the fusion proteins to bind to the resin. After 30 minutes, the resin was separated from the mixture via 5 minutes of centrifugation at 500 × *g* and 4°C. The resin was collected and washed twice with PBS, at 10 times the bed volume. The rHLT-B was recovered from the resin after the cleavage of the bound fusion protein with 50 units of thrombin per ml of bed volume, for 16 hours at room temperature. The suspension was then centrifuged (500 × *g*) at room temperature for 5 minutes, in order to pellet the Sepharose 4B beads. The supernatant harboring the target proteins was subsequently harvested, and the sample was filtered through a 0.22 μm syringe filter into a recovery solution containing rHLT-B, and any residual Sepharose was removed.

The ganglioside binding assay of the purified rLTB

GM1-ELISA was conducted in an attempt to determine the degree of affinity of the rHLT-B protein for the GM1-ganglioside receptor. Microtiter plates (96 wells) were coated with monosialoganglioside-GM1 (Sigma, USA) via incubation with GM1 (3 μg/ml, 100 μl per well) in bicarbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6) overnight at 4°C. Negative control wells were coated with BSA (3.0 μg/ml, 100 μl per well). Plates coated with monosialoganglioside-GM1 or the negative control, BSA, were incubated with increasing concentrations of the soluble purified rHLT-B proteins in bicarbonate buffer (100 μl per well) or with bacterial CT-B (positive control) (Sigma, USA) for 2 hours at 37°C, in order to allow for binding to occur between GM1 and rHLT-B. After incubation with rabbit anti-CT-B (1/8000 in PBS, Sigma, USA), all wells were incubated with 1 : 10,000 dilutions of goat anti-rabbit HRP (horseradish peroxidase) labeled IgG (Sigma, USA) in 0.01M PBS containing 0.5% BSA at 37°C, for 2 hours. The wells were subsequently washed three times with PBST. The development step was then conducted using p-nitrophenyl phosphate (Sigma, USA) as a chromogen. The absorbance values were measured at 405 nm, with a reference wavelength of 550 nm.

Analytical techniques

SDS-PAGE was conducted in accordance with the standard method (Sambrook and Russel, 2001). The soluble supernatant of the *E. coli* BL21 (DE3) cell lysate was loaded onto 15.0% (w/v) polyacrylamide gel. The gels were stained with 0.1% (w/v) Coomassie's Brilliant Blue R-250. A Bio-Rad gel image system was used for the analysis of the SDS-PAGE gels. For Western blotting analysis, the proteins on the gels were transferred onto PVDF (polyvinylidene difluoride) membranes (Bio-Rad) in a semi-dry blotting apparatus (120 minutes at 120 mA). The membranes were then blocked with 0.3% (w/v) BSA in Tris-buffered saline (TBS, pH 7.4), after which the membranes were incubated with goat anti-CT-B antibody and rabbit anti-goat HRP IgG in turn, in accordance with the manufacturer's instructions. After the application Western Blotting Luminol Reagent (Santa Cruz Biotechnology), membranes evidencing a positive signal were exposed to films, which were developed using the CURIX HT-330U (AGFA, Germany) automatic developing system. The images from the gels and membranes

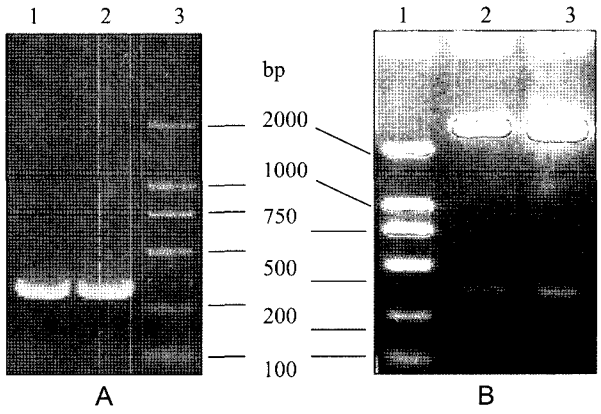

Fig. 2. Agarose-gel electrophoresis of PCR products and identification with restriction endonuclease. (A) Lane 1-2: PCR products using DNA from *E.coli* O6:H16 (LT^+, ST^+) as template, Pyrobest™ DNA as polymerase with primers Pb1 and Pb2. Lane 3: DNA marker. (B) Lane 1: DNA marker. Lane 2-3: Double restriction endonuclease *EcoR*I and *Not*I digestion of the recombinant plasmid pGEX-4T-1-rHLT-B.

were scanned with a GEL-DOC 2000 gel documentation system (Bio-Rad). Bradford protein assays were employed for the quantitative analyses of the target protein, following the manufacturer's instructions (Bradford, 1976.)

Results and Discussion

Construction of recombinant strain BL21 (DE3)/pGEX-4T-1-rHLT-B

The HLT-B gene was successfully cloned from *E. coli* O6:H16 with PCR. The pGEX-4T-1-rHLT-B expression vector was adequately constructed and fused with GST (Fig. 2), which functioned in this experiment as detection and purification tag. It was also used to enhance the soluble expression of the target protein (Frangioni and Neel, 1993). The HLT-B gene was under the tight control of the *tac* promoter, which was induced by the lactose analogue IPTG. A thrombin cleavage site was located between the C-terminals of the GST and HLT-B encoding sequences. The expression vector harboring the HLT-B gene was then transformed into *E. coli* BL21 (DE3). The resultant recombinant strain, designated BL21 (DE3)/pGEX-4T-1-rHLT-B, was then employed for the production of the target fusion protein.

Expression and detection of fusion protein GST- rHLT-B

BL21 (DE3)/pGEX-4T-1-rHLT-B was cultured in LB medium at 37°C, and induced with IPTG at a final concentration of 0.8 mM, in an attempt to characterize the production of the heterologous protein. Analysis of SDS-PAGE gels revealed that a foreign fusion protein had been expressed in soluble form, evidencing a molecular weight of 38.0 kDa. This was consistent with the theoretical molecular weight (Fig. 3). Western blotting analysis was employed in order to characterize the target protein in more detail.

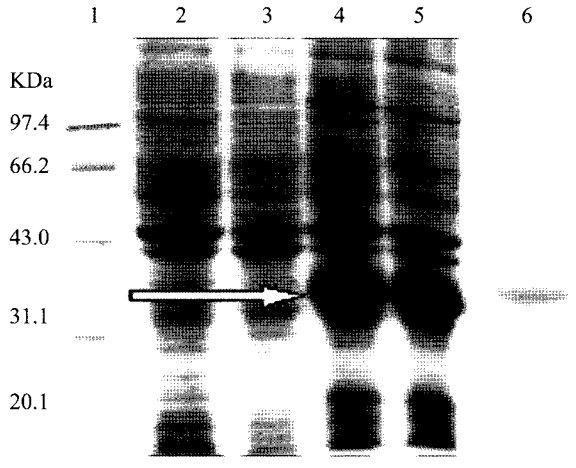

Fig. 3. SDS-PAGE and western blotting analysis of the fusion protein GST-rHLT-B.
Lane 1: protein molecular weight standards.
Lane 2-3: soluble proteins of *E.coli* BL21 (DE3) (negative control).
Lane 4-5: soluble protein of BL21 (DE3)/pGEX-4T-1-rHLT-B.
Lane 6: Western blotting film. The band corresponding to 38.0 kDa on the film is the fusion product GST-rHLT-B.

Optimization of Medium and pH

The process of recombinant strain fermentation was divided two distinct phases, namely the cell growth and foreign protein expression phases, in an effort to enhance plasmid stability and/or reduce the negative effects inherent to the expression of the heterologous protein on the host cells (Siva Kesava *et al.*, 1999; Gupta *et al.*, 2001). Generally, high cell density at the end of the cell growth phase tends to prove helpful for high-level expression, and the composition of the medium tends to be the primary factor that influences cell growth. Our comparison of cell growth profiles

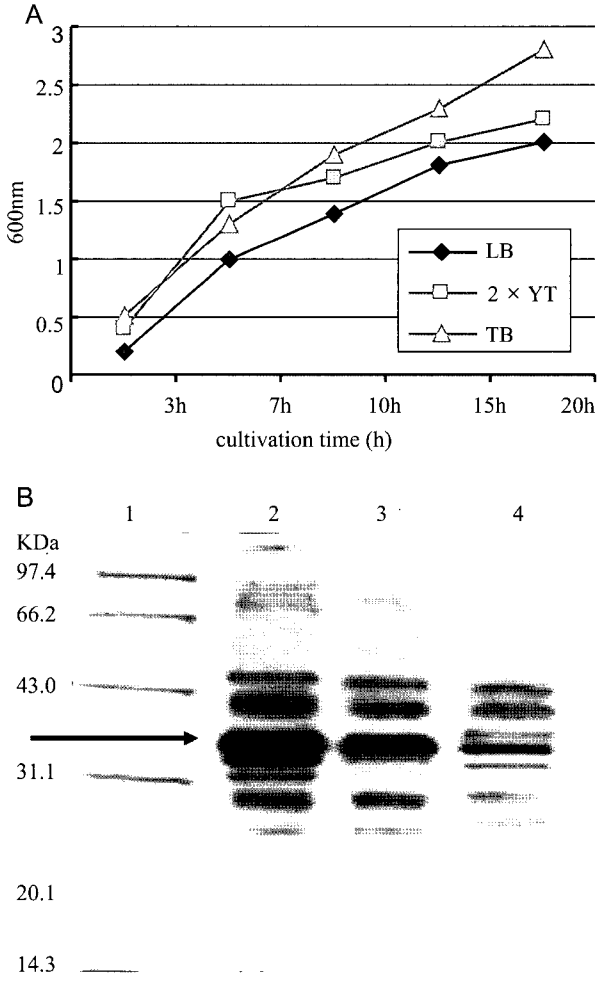

Fig. 4. (A) Growth profiles of BL21 (DE3)/pGEX-4T-1-rHLT-B cultivated in LB, 2 × YT and TB medium in shaking-flake fermentation. (B) Comparison of expression level of rHLT-B between three media by SDS-PAGE. Lane 1: protein molecular weight standards; Lane 2: TB medium; Lane 3: 2 × YT medium; Lane 4: LB medium.

and protein expression levels in the three media, via optical density measurements (A_{600}) and SDS-PAGE analysis, indicated that the highest BL21 (DE3)/pGEX-4T-1-rHLT-B cell density had been achieved in TB medium (Fig. 4). This suggested that rich nutrition proved favorable for cell growth. Therefore, all subsequent experiments utilized TB medium.

When the heterologous protein is expressed in the host cells, the pH of culture medium is known to be an important parameter, greatly affecting the yield of the soluble target protein. Different pH values were examined for the TB medium (6.0, 6.5, 7.0, 7.5, 8.0 and 8.5). Our results indicated that optimal target protein yield could be achieved at a pH of 8.0 (Table 1). The most reasonable explanation for this was that an alkaline medium was most capable of neutralizing the acids generated by *E. coli* in the course of metabolizing lactose as a partial carbon source (Xu *et al.*, 2000).

Optimal inducing temperatures, time, and lactose concentrations

Temperature is an important parameter in recombinant protein expression (Sambrook and Russel, 2001). Although comparable cell densities could be achieved at the stationary phase in a time range of 15-25 h at different temperatures, cell proliferation exhibited a slowdown at 25°C, as compared to what was observed under higher temperatures (37°C and 42°C). A comparison between the soluble fusion proteins expressed by BL21 (DE3)/pGEX-4T-1-rHLT-B in TB medium (pH 7.4) at 25, 30, 37 and 42°C revealed an interesting phenomenon. Along with in the expression temperature, the levels of GST-rHLT-B expression increased dramatically as the result of the addition of lactose (Table 2). These results showed that, at lower temperatures, both cell growth and protein synthesis occurred less rapidly. A higher solubility of the foreign proteins can be achieved by providing sufficient time and an optimal microenvironment to allow for them to fold into their native conformations (Bernardez and Clark, 1998).

The final lactose concentrations in the media also greatly affected the yield of soluble target proteins, and the most appropriate inducing concentrations were found to occur in a concentration range of 5-50 g/l. A final lactose concentration of 10 g/l was determined to be the optimal concentration with regard to soluble expression yield. The highest level of expression was achieved at 25°C, with a 5-hour culture time, using 10 g/l lactose as an inducer.

Table 1. Expression outputs of GST-rHLT-B in TB media under different pH in 3.7 L fermentor at 37°C

Expression outputs of GST-rHLT-B (% of total bacterial proteins)[a]						
pH	6.0	6.5	7.0	7.5	8.0	8.5
GST-rHLT-B	11.24	25.87	36.66	38.32	41.35	18.65

[a]The Expression outputs of fusion target protein was calculated as the ratio of band-intensity of "soluble fusion protein/total fusion protein". The band-intensity was analyzed by Quantity One software.

Table 2. Expression outputs of GST-rLTB proteins induced by different concentration of lactose and temperature

Concentrations of Inducer		Expression outputs of GST-rHLT-B[a] (% of total bacterial proteins)			
		25°C (5 h)	30°C (5 h)	37°C (5 h)	42°C (5 h)
Lactose (g/l)[b]	50	31.25	20.45	18.43	17.67
	10	38.66	25.24	22.46	20.21
	5	27.99	21.67	18.35	16.19
IPTG (mM)	0.5	10.25	12.58	19.35	15.87
	1.0	13.34	14.86	22.67	20.86

[a]The Expression outputs of fusion target protein was calculated as the ratio of band-intensity of "soluble fusion protein/total fusion protein". The band-intensity was analyzed by Quantity One software.
[b]Lactose at a final concentrations of 5, 10, and 50 g/l were added with dry powder at the same stage of the fermentation (A_{600}=0.6).

Effects of lactose on rHLT-B expression and purification of rHLT-B

IPTG is a highly stable and effective inducer of the *lac* promoter, as well as its derivatives, including the *tac* promoter, in the expression of target recombinant proteins. IPTG is extensively employed in laboratories. However, the use of IPTG as an inducer in the industrial production of engineering products is rather limited, due primarily due to its potential toxicity and high price (Kilikian *et al.*, 2000). In this study, the use of the relatively inexpensive and non-toxic lactose instead of IPTG as the inducer, was found to be quite advantageous with regard to the expression of soluble recombinant proteins under optimal fermentation conditions. The yield of soluble GST-rHLT-B was 2-fold higher than that in IPTG-inducing expression system. Glutathione-Sepharose 4B affinity chromatography has been designed for the rapid single-step purification of soluble recombinant GST fusion proteins. rHLT-B was recovered from the resin via the cleavage of the bound fusion protein, GST-rHLT-B, by the addition of thrombin (Fig. 5).

Biological characterization of purified rHLT-B

Ninety six mg of purified rHLT-B were obtained per liter of fermentation broth in a 3.7 L fermentor under

optimized fermentation conditions, via the addition of suitable concentrations of lactose as an inducer. The ability of the purified rHLT-B to bind to the GM1 ganglioside was evaluated in our series of ELISA experiments. The GM1 ganglioside-binding capability of the purified rHLT-B was also determined using BSA as a negative control, and the commercially available cholera toxin B subunit (CT-B) as a positive control. The results of these trials demonstrated that rHLT-B bound preferentially to GM1. This degree of binding affinity was comparable to that associated with CT-B (Fig. 6).

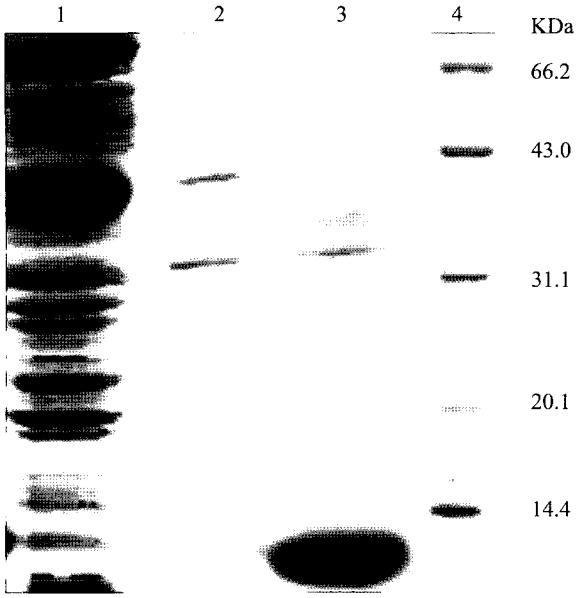

Fig. 5. SDS-PAGE analysis of purification of rHLT-B.
Lane 1: total protein extracted from *E. coli* BL21 (DE3)/pGEX-4T-1-rHLT-B.
Lane 2: the fraction eluted with 10 bed volumes of PBS.
Lane 3: purified rHLT-B sample from GST affinity chromatography after the cleavage of 50 units of thrombin.
Lane 4: protein molecular weight standards.

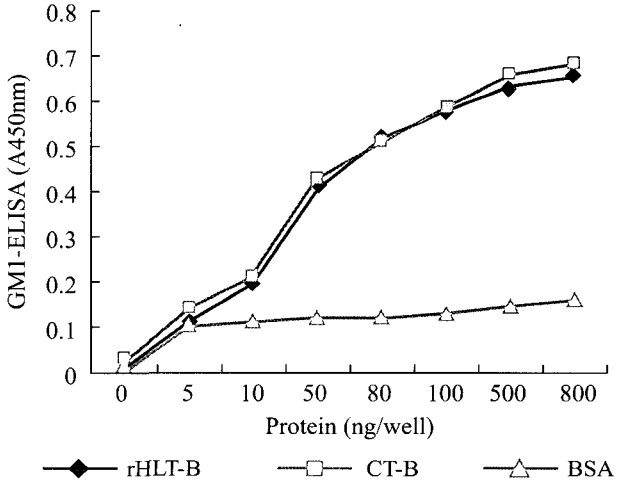

Fig. 6. Capacity of rHLT-B binding GM1 ganglioside. The ELISA experiment was performed by incubation of coated GM1 ganglioside with purified rHLT-B or with commercially available CT-B as positive control and BSA as negative controls.

Conclusion

The GST-rHLT-B recombinant protein was expressed in *E. coli* in its soluble form. Expression levels of up to more than 38% of total cellular proteins were achieved using the following tactics: use of a functional fusion tag, optimization of culture conditions, and the use of lactose rather than IPTG as an inducer. The purity of the target protein, rHLT-B, was up to 94% (w/w) after a single-step GST-affinity chromatography, coupled with thrombin-induced cleavage. A yield of rHLT-B of up to 96 mg/l of fermentation broth was achieved. The percentage of soluble recombinant rHLT-B increased 3 times than registered prior to optimization. This result clearly indicates that the expression of the fusion protein under optimized culture conditions, with lactose used as an inducer facilitated the high-level expression of soluble rHLT-B in *E. coli*. This conclusion provides us with some insight into the possible large-scale production protocols for other promising peptides in the enterotoxin family.

Acknowledgement

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