Effects of lactose as an inducer on expression of Helicobacter pylori rUreB and rHpaA, and Escherichia coli rLTKA63 and rLTB

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Abstract
AIM: To demonstrate the effect of lactose as an inducer on expression of the recombinant proteins encoded by Helicobacter pylori ureB and hpaA, and Escherichia coli LTB and LTKA63 genes and to determine the optimal expression parameters.

METHODS: By using SDS-PAGE and BIO-RAD gel image analysis system, the outputs of the target recombinant proteins expressed by pET32a-ureB-E.coliBL21, pET32a-hpaA-E.coliBL21, pET32a-LTKA63-E.coliBL21 and pET32a-LTB-E.coliBL21 were measured when using lactose as inducer at different dosages, original bacterial concentrations, various inducing temperatures and times. The results of the target protein expression induced by lactose were compared to those by isopropyl-β-D-thiogalactoside (IPTG). The proteins were expressed in E.coli.

RESULTS: Lactose showed higher efficiency of inducing the expression of rHpaA, rUreB, rLTB and rLTKA63 than IPTG. The expression outputs of the target recombinant proteins induced at 37 °C were remarkably higher than those at 28 °C. Other optimal expression parameters for the original bacterial concentrations, dosages of lactose and inducing time were 0.8, 50 g/L and 4 h for rHpaA; 0.8, 100 g/L and 4 h for rLTKA63; 1.2, 100 g/L and 5 h for both rUreB and rLTB, respectively.

CONCLUSION: Lactose, a sugar with non-toxicity and low cost, is able to induce the recombinant genes to express the target proteins with higher efficiency than IPTG. The results in this study establish a beneficial foundation for industrial production of H pylori genetic engineering vaccine.

INTRODUCTION
In China, chronic gastritis and peptic ulcer are two most common gastric diseases, and gastric cancer is one of the malignant tumors with high mortalities and morbidities. Helicobacter pylori (H pylori), a microaerophilic, spiral and Gram-negative bacterium, is recognized as a human-specific gastric pathogen that colonizes the stomachs of at least half of the world’s populations. Most infected individuals are asymptomatic. However, in some subjects, H pylori infection causes acute, chronic gastritis and peptic ulceration, and acts as a high risk factor on development of gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma.

It is generally considered inoculation of H pylori vaccine is a most efficient measure for prevention and control of H pylori infection. However, high nutrition requirements, poor growth for a long time, easy contamination during the cultivation and difficulty of bacterial strain conservation make whole cell vaccine of H pylori impracticable. Genetic engineering vaccine seems to be a possible pathway for developing H pylori vaccine.

Isopropyl-β-D-thiogalactoside (IPTG), a highly stable and effective inducer on T7lac promoter for target recombinant protein expression, is widely used in laboratories. However, IPTG is a reagent with potential toxicity and high cost, which limited it as a practical inducer for industrial production of genetic engineering vaccines. It was reported that lactose, a common disaccharide, is also able to induce T7lac promoter after it is transformed into allo lactose. The low-cost and non-toxicity make lactose a practical potential for engineering products. In comparison with IPTG, the parameters of lactose inducing different recombinant protein expression vary greatly and its optimal working conditions are established usually by a large number of laboratory tests. In our previous studies, urease subunit B (UreB) and H pylori adhesin (HpaA) were demonstrated as fine candidates in H pylori engineering vaccine. For improving immunogenicity of the vaccine, heat-labile enterotoxin subunit A mutant at the 63rd position (LTKA63) and subunit B (LTB) of Escherichia coli were selected as adjuvants. In this study, 4 constructed prokaryotic expression systems of ureB, hpaA, LTKA63 and LTB were used as the target genes to determine the inducing effects with different lactose dosages, temperatures and times, and original bacterial concentrations on expression of the recombinant proteins.

MATERIALS AND METHODS

Materials
Four prokaryotic expression systems of pET32a-ureB-E.coliBL21, pET32a-hpaA-E.coliBL21, pET32a-LTKA63-E.coliBL21 and pET32a-LTB-E.coliBL21 were constructed and offered by our laboratory. Tryptone, yeast extract for LB medium were purchased from OXOID (Basingstoke, Hampshire, England). IPTG and lactose used for inducement, and SDS, glycine and DTT were offered by BBST (Shanghai, China). Acrylamide, N,N’-methylene-bis-acrylamid and TEMED were obtained from Serva (Heidelberg, Germany).

Methods
Determination of optimal inducing concentrations of lactose
and temperatures. A colony of each of the four engineering bacteria in LB agar plates was inoculated into 5 mL of LB liquid medium and then incubated on rotator with 200 r/min at 37 °C for 12 h. The values at A_{600} were measured by spectrophotometry to indicate the bacterial concentrations. Lactose at the final concentrations of 5, 10, 50 and 100 g/L were added into the cultures of the 4 strains with the A_{600} values of 1.2, respectively, and then incubated on 200 r/min shaking at 37 °C or 37 °C for 4 h. The bacteria in the medium were collected by centrifugation.

Expressing rUreB, rHpaA, rLTKA63 and rLTB were examined and estimated, respectively. BIO-RAD gel image analysis system was applied to measure the outputs of target protein fragments by their area percentages in the total bacterial proteins. 0.5 mmol/L IPTG was simultaneously used as an inducer control, which inducing effects for the four recombinant proteins had been confirmed in our previous studies.

### Table 1: Expression outputs of rUreB, rHpaA, rLTKA63 and rLTB proteins induced by different concentrations of lactose and IPTG

| Lactose (g/L) and IPTG (mmol/L) | rUreB (% of total bacterial proteins) | rHpaA (% of total bacterial proteins) | rLTKA63 (% of total bacterial proteins) | rLTB (% of total bacterial proteins) |
|---------------------------------|--------------------------------------|--------------------------------------|----------------------------------------|--------------------------------------|
|                                 | (37 °C)                              | (28 °C)                              | (37 °C)                                | (28 °C)                              |
| Lactose (50)                    | 40.59                                | 26.38                                | 50.35                                  | 44.72                                | 23.36                                | 22.41                                | 39.55                                | 21.91                                |
| (10)                            | 50.52                                | 39.70                                | 57.61                                  | 44.86                                | 35.84                                | 27.09                                | 42.66                                | 26.17                                |
| (5)                             | 30.51                                | 22.17                                | 60.80                                  | 46.42                                | 35.64                                | 22.09                                | 26.99                                | 23.94                                |
| (1)                             | 27.33                                | 18.92                                | 54.00                                  | 50.29                                | 21.91                                | 21.73                                | 23.33                                | 10.65                                |
| (0.5)                           | 20.78                                | 13.63                                | 42.29                                  | 36.05                                | 22.01                                | 12.24                                | 14.72                                | 10.31                                |
| IPTG (0.5)                      | 16.66                                | 14.72                                | 30.65                                  | 22.61                                | 16.75                                | 14.48                                | 12.36                                | 10.06                                |

Effects of the target protein expression by using different inducing time

According to the results obtained above, the optimal original bacterial concentration for inducement was 1.2 (A_{600}). The four different bacterial cultures (A_{600}=1.2), which expressing rUreB, rHpaA, rLTKA63 or rLTB, were added with lactose at the final concentrations of 100, 50, 100 and 100 g/L for inducement of rUreB, rHpaA, rLTKA63 and rLTB, respectively. The lactose added cultures were continuously incubated on lactose at the final concentrations of 5, 10, 50 and 100 g/L for inducement of rUreB, rHpaA, rLTKA63 and rLTB. Lane 2: Induced with 100 g/L lactose; Lane 3: Induced with 50 g/L lactose; Lane 4: Non-induced. C: rLTKA63; Lane 1: Marker; Lane 4: Non-induced. D: rLTB; Lane 1: Marker; Lane 2: Induced with 0.5 mmol/L IPTG; Lane 3: Induced with 100 g/L lactose. Lane 4: Non-induced.

Effects of lactose on inducing expression of target recombinant proteins compared to IPTG. A: rUreB; Lane 1: Marker; Lane 2: Induced with 0.5 mmol/L IPTG; Lane 3: Induced with 100 g/L lactose; Lane 4: Non-induced. B: rHpaA; Lane 1: Marker; Lane 2: Induced with 0.5 mmol/L IPTG; Lane 3: Induced with 50 g/L lactose; Lane 4: Non-induced. C: rLTKA63 expression; Lane 1: Marker; Lane 2: Induced with 0.5 mmol/L IPTG; Lane 3: Induced with 100 g/L lactose. Lane 4: Non-induced. D: rLTB; Lane 1: Marker; Lane 2: Induced with 0.5 mmol/L IPTG; Lane 3: Induced with 100 g/L lactose; Lane 4: Non-induced.

Effects of the target protein expression of original bacteria with different A_{600} values under inducement by lactose

By using the inducing concentrations of lactose with 100, 50, 100 and 100 g/L inducing at 37 °C for 4 h, the expression outputs of the 4 recombinant proteins with the different original bacterial concentrations (0.2-2.4 A_{600} values) are showed in Figure 2. The results indicated that a better expression effect for any of the 4 recombinant proteins was present when the original bacterial concentration used was higher (A_{600}=0.8-1.2).
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Effects of the target protein expression using different inducing time

When the original bacterial concentrations as $A_{600}$ values of 1.2 and the lactose concentrations of 100, 50, 100 and 100 g/L, the outputs of rUreB, rHpaA, rLTKA63 and rLTB were shown in Figure 3. The results indicated that a higher output for any of the 4 recombinant proteins was present when the inducing time was 4-5 h.

DISCUSSION

H pylori causes a local superficial infection in human stomach and duodenum[22]. So orally inoculating of H pylori vaccine has a good protective effect[23]. So far, no commercial H pylori engineering vaccine has been available. An engineering vaccine has many advantages but its immunoprotective effect is usually poor because of the unitarity for antigen components. Using adjuvant is an efficient measure to improve immune effect of engineering vaccines[24]. UreB and HpaA were demonstrated to be excellent antigen candidates as their stable and high expression, strong antigenicity, universal distribution in different H pylori isolates and exposure on the surface of the bacteria[25-28]. E. coli LTKA63 and LTB were recently found and were generally considered as the most efficient adjuvants for mucosal immunization so far[27,28]. So we planned to use the rUreB and rHpaA as antigens and LTB or LTKA63 as adjuvants to develop an oral taken double-valence genetic engineering vaccine of H pylori.

Almost of all the recombinant proteins show a very low expression or non-expression without inducement. IPTG is a routinely laboratory used inducer with high efficiency on recombinant protein expression in E. coli but it must be removed by complicated methods from the induced products because of its toxicity. When at the efficient inducing dosages, the cost of IPTG is approximate hundredfold of lactose. Therefore, lactose as an inducer has industrially remarkable advantages. Lactose, differs from IPTG, is unable to enter the bacterial body. It must be helped by a special enzyme called as primase to be transported into host bacterium. The lactose in bacterial cell must be transferred into allo-lactose by 3-galactosidase and the latter is able to start T7 Lac promotor. In the process inducing expression of recombinant protein, lactose is much more complex than IPTG. So lactose, if used it as an efficient inducer, must be clarified its inducing parameters such as dosage, temperature, time and original bacterial concentration.

It was proved by our study that lactose at the multiple tested concentrations could efficiently induce the expression of rUreB, rHpaA, rLTKA63 and rLTB. The effects of lactose on inducing expression of the 4 recombinant proteins were much stronger than that by IPTG, which demonstrated by 98.4-245.1% increased outputs (Table 1). Furthermore, it was reported that lactose is a carbon source for bacteria to promote growth and increase number of bacteria in culture, which results in the increase of output of the target recombinant protein.

The results of this study indicated that at 37 °C for 4 h inducement the lactose concentration to obtain the highest expression outputs of rUreB, rHpaA, rLTKA63 and rLTB were 100, 50, 100 and 100 g/L, respectively. It was found in the study that inducing temperatures can obviously affect the expression of the recombinant proteins. For example, with the original bacterial concentration of 1.2 $A_{600}$ value for 4 h inducement, using 37 °C as the inducing temperature could increase the expression outputs of rUreB, rHpaA, rLTKA63 and rLTB with 27.3-53.9%, 7.4-30.9%, 0.8-79.8% and 12.7-119.1% of those induced by 28 °C. The original bacterial concentrations when lactose addition was found to affect the outputs of the recombinant proteins. There were preferable expression effects of the recombinant proteins to add lactose when the original bacterial concentrations with the $A_{600}$ values of 0.8-1.2. With the original bacterial concentration of 1.2 $A_{600}$ values and at temperature 37 °C, the outputs of the recombinant proteins for 4-5 h inducement by the optimal concentrations of lactose were relatively high.

Summarily, using lactose as an inducer on the expression of pET32a-ureB-E.coli BL21, pET32a-hpaA-E.coli BL21, pET32a-LTKA63-E.coli BL21 and pET32a-LTB-E.coli BL21, the optimal temperature was 37 °C. The rest optimal parameters for the original bacterial concentrations, dosages of lactose and inducing time were 0.8, 50 g/L and 4 h for rHpaA; 0.8, 100 g/L and 4 h for rLTKA63; 1.2, 100 g/L and 5 h for both rUreB and rLTB, respectively. The results from this study established a beneficial foundation for industrial production of H pylori genetic engineering vaccines.

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