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Notes & Tips

Culture of *Escherichia coli* in SOC medium improves the cloning efficiency of toxic protein genes

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ABSTRACT

In this paper, we report a useful protocol for cloning toxic protein genes. Use of the SOC medium, which is a glucose-containing rich medium, significantly improved the transformation efficiency of a recombinant plasmid containing a toxic plant subtilase *SaSBT1* cDNA. Both glucose and rich nutrients present in the SOC medium prevented the unintended activation of the *lac* promoter carried on the cloning vector, and led to significantly improved transformation efficiency of recombinant plasmids containing toxic protein genes and an increased rate of transformant growth.

Recombinant plasmids containing toxic protein genes are prone to instability and rejection by bacterial host *Escherichia coli* strains, resulting in severely reduced transformation efficiency and accumulated mutations [1–3]. Although several alternative approaches have been proposed to overcome the above problems, such as cell-free cloning technology [2], conventional *E. coli*-based cloning remains one of the most widely employed protocols in molecular biology. The most widely used commercial protein expression vectors employ *lac* or *lac*-T7 coupling promoters that drive the expression of a downstream coding sequence and are induced by the addition of the lactose analogue IPTG (isopropyl β-D-1- thiogalactopyranoside).[2] However, relatively high background expression is often detected in these expression systems in the absence of induction [4].

Previously, we attempted to clone a plant subtilisin-like protease SaSBT1 cDNA from *Solanum americanum*, and heterogeneously express it in *E. coli*. The RT-PCR product with intact *SaSBT1* ORF, purified from an agarose gel, was ligated into the pGEM-T Easy vector (Promega) and then transformed into the competent *E. coli* cells (strain TOP10) prepared according to the method described by Mande and Higa [5]. Unfortunately, only blue colonies (empty vector transformants) were obtained. We repeated the experiment several times with unsatisfactory results. In contrast, separation of the intact coding sequence of SaSBT1 protease into two fragments (encoding N-terminal and C-terminal fragments of SaSBT1, respectively) permitted successful cloning using the pGEM-T Easy vector. We therefore hypothesized that the *lac* promoter might have been induced by IPTG, which was used for blue/white screening during the above-described transformations, and that expression of the fusion protein was harmful to the bacterial host strains. Therefore, we eliminated IPTG and X-gal (5-bromo-4-chloro-3-indolyl β-D-galactoside) from the LB (Luria Bertani broth) solid medium and verified positive colonies by both colony PCR and restriction enzyme digestion analysis of plasmids. The results of these analyses indicated that all colonies contained the empty pGEM-T Easy vector lacking the cDNA insert.

Basal or leaky expression of the toxic protease SaSBT1 driven by the *lac* promoter may be responsible for our difficulty in obtaining positive transformants. This phenomenon has been previously reported [3]. Studies [4] suggested that small amounts of lactose contaminants in the medium were sufficient to induce the *lac* promoter activity. Peptone/tryptone and yeast extract are both used in some growth media, including LB. If either of these mixtures contains trace amounts of lactose, the *lac* promoter would certainly be activated. Since glucose may prevent the induction of the *lac* promoter by lactose [4], we replaced LB medium [6] (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl pH 7.0) with glucose-containing SOC medium [6] (20 mM glucose, 20 g/L tryptone, 5 g/L yeast extract, 0.5 g/L NaCl, 2.5 mM KCl, 5 mM $MgCl_2$, and 5 mM $MgSO_4$, pH 7.0) in all stages of *E. coli* culture to avoid unintended activation of the *lac* promoter. As expected, by using the glucose-containing SOC medium, we successfully obtained positive colonies harboring recombinant plasmid pDLW2 containing a *SaSBT1* cDNA in pGEM-T Easy vector.

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[2] *Abbreviatons used*: IPTG, isopropyl β-D-1-thiogalactopyranoside; LB, Luria Bertani broth; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

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Our subsequent growth curve experiments (Fig. 1) demonstrated that the key to overcoming the toxic effect of the recombinant SaSBT1 was the use of glucose and rich nutrients present in SOC medium. The bacteria harboring pDLW2 grew slowly and reached the stationary phase at OD_{600} of ca. 0.76 after 12 h in LB medium (Fig. 1A). When 20 mM glucose was added to LB medium, however, the bacteria grew faster and reached an OD_{600} of ca. 1.29 after 12 h (Fig. 1A). In nutrient-rich and glucose-containing SOC medium, it took only 8 h to reach an OD_{600} of ca. 0.95 (Fig. 1A). In SOB medium, which is identical to SOC medium, except that it does not contain 20 mM glucose [6], the bacteria grew slightly slower than in SOC medium before 8 h (Fig. 1A). In contrast, the bacteria harboring the control vector pGEM-T Easy showed similar growth rates in LB medium and in LB medium plus 20 mM glucose, although the cells also grew faster in SOC and SOB medium (Fig. 1B).

Unexpectedly, after 8 h, in SOB medium both bacteria harboring pDLW2 (Fig. 1A) or the control vector pGEM-T Easy (Fig. 1B) continued to grow well until 12 h, whereas in SOC medium the bacterial growth was slowed significantly. To examine possible causes for the inhibition of the bacterial growth in SOC medium, we determined the pHs of the bacterial cultures after 20 h culture with different media, and found that the pHs of the two glucose-containing media (LB + glucose and SOC) had much lower pH values (ca. 5) than those of the two media without glucose (LB and SOB, ca. 8.5) (Fig. 1). It has been suggested that glucose in the medium may produce acid metabolites during the bacterial growth [4], which result in the low pH value of the culture, and therefore would be responsible for the inhibition of the bacterial growth.

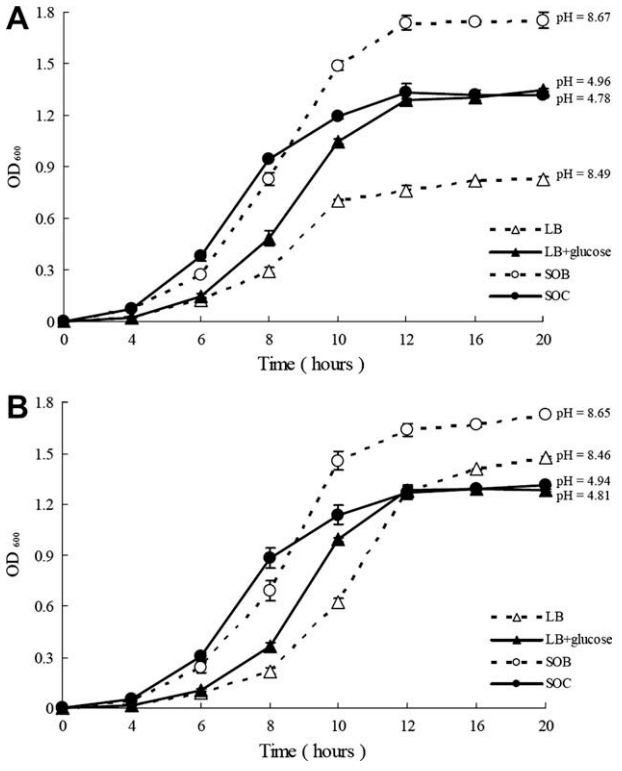

Fig. 1. Growth curves of the *E. coli* cells harboring pDLW2 containing plant subtilase *SaSBT1* cDNA and cloning vector pGEM-T Easy. Two microliters of overnight grown bacteria containing recombinant pDLW2 (A) or control vector pGEM-T Easy (B) were inoculated into 20 ml of LB, LB plus 20 mM glucose, SOB, and SOC medium, respectively, and cultured at 37 °C with shaking. The pH values of the cultures after 20 h culture with different media are shown beside the growth curves.

The suppression of the *lac* promoter activity by glucose present in growth medium was further confirmed by the expression analysis of *lacZ* (β-galactosidase) gene carried on the pGEM-T Easy cloning vector in different media with or without glucose (Figs. 2A–D). The colonies grown on LB medium are dark blue (Fig. 2A), whereas the colonies grown on the LB medium plus 20 mM glucose show only faint blue (Fig. 2B). Similarly, the colonies grown on SOB medium are light blue (Fig. 2C), whereas the colonies grown on the SOC medium are white (Fig. 2D). This observation is supported by our quantitative analysis of the β-galactosidase activity of cells grown in the four media (Fig. 2E). The addition of glucose to the LB medium significantly reduced the β-galactosidase activity from 818.46 nM/min/mg (LB) to 46.17 nM/min/mg (LB + glucose). The inclusion of glucose in the nutrient-rich medium also greatly inhibited the β-galactosidase activity, i.e., from 286.96 nM/min/mg (SOB) to 11.06 nM/min/mg (SOC). It is worth noting that the β-galactosidase activity of cells grown in SOB medium was about 1/3 of that in LB medium (Fig. 2E), indicating that rich nutrients other than glucose could also prevent the expression of *lacZ* in SOC medium. This result is consistent with the notion that a nutrient-rich medium provides an added safeguard against unintended *lac* induction because nutrient limitation can lead to cAMP accumulation, which then leads to *lac* promoter activation [3]. For this reason, we chose to use SOC medium rather than supplementing LB medium with glucose. We observed that use of SOC medium resulted in significantly faster growth of *E. coli* in relation to growth in LB medium. This increase in growth rate translated to the production of larger and more readily visible colonies on the SOC plate within 12 h; notably, only small colonies were visible on the LB plate after overnight growth (16–20 h) (Supplemental Fig. 1).

We also observed enhanced transformation efficiency when using SOC medium in all steps in contrast to the standard protocol, which uses SOC medium only in the heat shock recovery step [7]. By the transformation procedure of Cohen et al. [7,8] or Inoue et al. [7,9], the transformation efficiency reached 8.27×10^7 cfu/μg DNA and 1.04×10^8 cfu/μg DNA when the bacteria were grown in SOC medium, but only at 3.40×10^7 cfu/μg DNA and 3.33×10^7 cfu/μg DNA in LB medium, respectively (Supplemental Table 1). Cohen's method used in our experiments was slightly modified in which the diluted (1:100) *E. coli* were cultured at 18–20 °C rather than 37 °C, which resulted in a somewhat higher transformation efficiency than the efficiency reported [7]. The rationale behind our modification is based on the Inoue discovery [9] that low temperature cultivation can improve the transformation efficiency of competent cells. Our optimized transformation protocol using SOC medium is described in the Supplemental materials.

Although transformation efficiency and bacteria growth are improved by the use of SOC medium, there are drawbacks to using this medium for *E. coli* transformation. To some extent, it conflicts with the blue/white screening system. The use of SOC medium turns off the *lac* promoter and prevents the expression of toxic fragments and β-galactosidase, thus rendering all the colonies white on the first day of growth. However, false colonies turn blue on the next several days when glucose is depleted.

In conclusion, we provide experimental evidence that growth of *E. coli* in glucose-containing SOC medium leads to significantly improved transformation efficiency of recombinant plasmids containing toxic protein genes and an increased rate of cell growth. We strongly recommend the use of nutrient-rich SOC medium to culture *E. coli* in all cloning steps of genes encoding toxic proteins.

Acknowledgments

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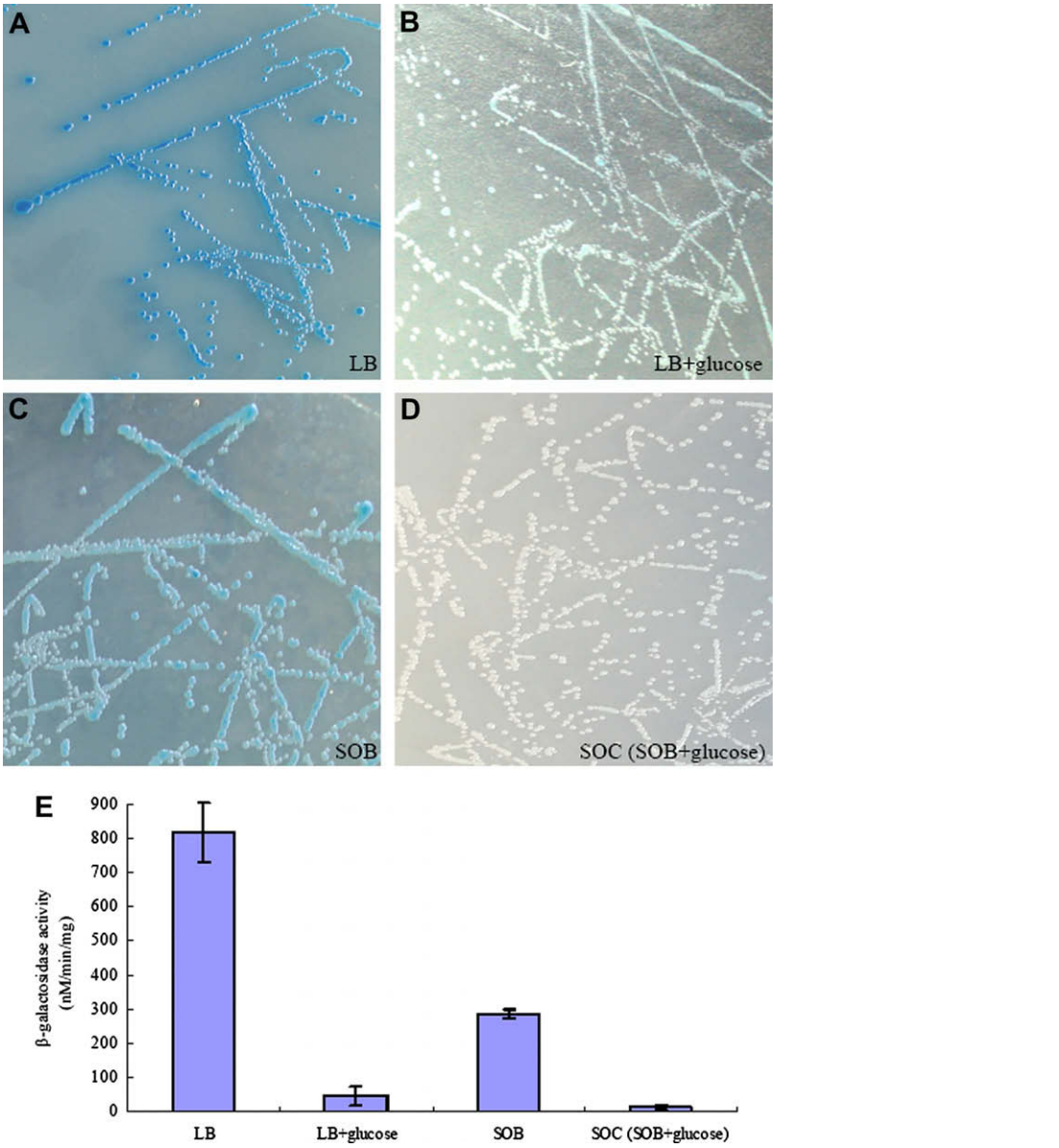

Fig. 2. Expression of *lacZ* (β-galactosidase) gene carried on the pGEM-T Easy cloning vector in different media with or without glucose. *E. coli* (strain TOP10) cells containing pGEM-T Easy were grown for 24 h at 37 °C on the LB (A), LB + 20 mM glucose (B), SOB (C), and SOC (D) medium, respectively. Forty microliters of 2% X-gal were spread on the surface of each plate (without IPTG). (E) Quantitative analysis of the β-galactosidase activity of cells grown in the four media using the Bacterial GAL Colorimetric Assay Kit (GMS600003.3 from GenMed Scientifics Inc., Westbury, NY, USA).

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Appendix A. Supplementary data

Supplementary data for this article may be found in the online version at doi:10.1016/j.ab.2009.07.023.

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