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ORIGINAL PAPER

High cell density cultivation of *Escherichia coli* with surface anchored transglucosidase for use as whole-cell biocatalyst for α-arbutin synthesis

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Abstract A fed-batch culture strategy for the production of recombinant *Escherichia coli* cells anchoring surface-displayed transglucosidase for use as a whole-cell biocatalyst for α-arbutin synthesis was developed. Lactose was used as an inducer of the recombinant protein. In fed-batch cultures, dissolved oxygen was used as the feed indicator for glucose, thus accumulation of glucose and acetate that affected the cell growth and recombinant protein production was avoided. Fed-batch fermentation with lactose induction yielded a biomass of 18 g/L, and the cells possessed very high transglucosylation activity. In the synthesis of α-arbutin by hydroquinone glucosylation, the whole-cell biocatalysts showed a specific activity of 501 nkat/g cell and produced 21 g/L of arbutin, which corresponded to 76% molar conversion. A sixfold increased productivity of whole cell biocatalysts was obtained in the fed-batch culture with lactose induction, as compared to batch culture induced by IPTG.

Keywords *Escherichia coli* · Surface-display · Transglucosidase · Induction · Fed-batch

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Introduction

Arbutin is a hydroquinone glucoside that is widely distributed in various plants of Ericaceae such as *Arctostaphylos uva-ursi* and *Vaccinium* spp. It inhibits tyrosinase activity in melanin synthesis by slowly releasing the active component, hydroquinone, through hydrolysis of its glucoside group [5, 8, 16]. It is a safe and mild agent for treating hyperpigmentation disorders compared to hydroquinone and has immense market potential in cosmetics industry. Structurally, α-arbutin is an α-glucoside of hydroquinone and the α-glucosidic bond offers higher stability and efficacy on melanogensis than the β-form. [5, 24, 25]. Due to its better mode of inhibition of tyrosinase, production of α-arbutin has been investigated by several researchers. α-Arbutin is produced by the glucosylation of hydroquinone in the presence of purified enzymes [13, 18] or by whole microbial [14] or plant cell biocatalysis [9, 15, 27]. The use of highly purified enzymes in the production process will ultimately result in very expensive α-arbutin, and whole cells usage results in lower productivities due to mass transfer limitations imposed by the cellular membrane.

Development of whole-cell biocatalysts displaying the target enzyme directly on the cell surface is one of the methods of overcoming substrate permeation barrier and to improve product yields. For example, using *Pseudomonas syringae* ice nucleation protein (INP) as a carrier protein, enzymes such as levansucrase [10], carboxymethylcellulase [11], and organophosphorus hydrolase [23] have been successfully displayed on *Escherichia coli* surface for use as whole-cell biocatalysts in the related applications. In the previous study [26], we have successfully constructed engineered *E. coli* displaying transglucosidase on the cell surface using INP anchoring motif. The whole cells expressing INPNC-transglucosidase at surface with very

high transglucosylating activity were applied as biocatalysts in the glucosylation of hydroquinone with maltose as the sugar donor, and a high yield of arbutin was obtained [26].

In this study, the cost effective production of large amount of engineered *E. coli* cells anchoring surface-displayed transglucosidase for biotransformation by fed-batch fermentations was demonstrated. The cell concentration and the transglucosylation activity of the whole-cell biocatalyst for arbutin synthesis were used as the parameters for analyzing the results of this study.

Materials and methods

Strain, plasmid and culture media

Escherichia coli BL21 (λDE3) [F⁻ *hsdS gal* (*λcIts857 ind1 Sam7 nin5 lacUV5-T7 gene 1*)] was transformed with plasmid pInaXNC1-aglA for the expression of INPNC-transglucosidase fusion protein [26]. The growing *E. coli* cells were induced with IPTG or lactose to express the fusion protein. The recombinant cells were maintained on LB-kanamycin (LB-Kan) agar plates for short-term storage. For long-term storage, the cells were frozen in 20% glycerol at −20°C.

The composition of fermentation media was based on the recipe summarized in Table 1. Trace metal solution was made according to Shiloach and Bauer [21] with the following modification in composition (g/L): $FeCl_3 \cdot 6H_2O$, 27; $ZnCl_2 \cdot 4H_2O$, 2; $CoCl_2 \cdot 6H_2O$, 2; $Na_2MoO_4 \cdot 6H_2O$, 2; $CaCl_2 \cdot 2H_2O$, 1; H_3BO_3, 0.5, and 100 mL/L HCl. To maintain selection pressure, kanamycin (30 μg/mL) was added to the preculture and fermentor at the beginning of cultivation. After sterilizing the contents of the fermentor, a mixture of $MgSO_4 \cdot 7H_2O$, kanamycin, trace metal solution, and glucose was added for batch fermentation.

Inoculum preparation and fermentation

For inoculum, a single colony of recombinant *E. coli* from a freshly prepared LB-Kan agar plate was inoculated into 50 mL of LB-Kan medium in a 250 mL baffled Erlenmeyer flask, and cultivated at 37 °C and 200 rpm for 15–18 h. Cultures grown to an optical density (OD_{600}) of 2–2.5 were used to inoculate growth media in shake flask or fermentor. All cultures were initiated close to an OD_{600} of 0.15 in batch and fed-batch cultures.

Batch cultures of recombinant *E. coli* were carried out by inoculating 3.3% of seed culture into 3 L of growth medium in a 5 L stirred tank fermentor (BIOSTAT-A, B. Braun Biotech International, Germany) and cultivating at 30 or 37 °C and 600 rpm. The air flow was maintained at 1 vvm. The pH was controlled at 7.0 by the addition of 2 N NaOH. The fed-batch cultures were initiated as batch cultures using medium B. The batch cultures were converted to fed-batch by sequential feeding of two media, F1 and F2, and the volumes of F1 and F2 used were as shown in Table 1. For fed-batch culture, the dissolved oxygen (DO) concentration was not allowed to fall below 30% of air saturation, initially by manually adjusting the agitation rate and then by mixing O_2 at a constant flow rate with air. Glucose was fed in response to the DO concentration. When the DO level rose above 40%, a pump was activated by an on–off time controller with step-change increase to deliver fresh medium containing 40% glucose (Table 1) to the culture and maintained glucose level in the culture broth at 0–0.5 g/L. There was an off–time of 60 sec to allow the DO to reestablish its value at 30% before the pump was activated again. For fusion protein expression, 50 mL of 0.5 M lactose solution was added to the culture by pulses. After commencing the induction phase, temperature of cultivation was shifted to 25 °C in 5 min and held at 25 °C for fusion protein expression.

Table 1 Medium composition

Component (g/L)	Medium					
	A	B	C	D	F1	F2
Glucose	–	2.0	2.0	2.0	400	–
Tryptone	10.0	10.0	10.0	10.0	–	–
Yeast extract	5.0	5.0	5.0	5.0	–	100
NaCl	5.0	5.0	5.0	5.0	–	–
K_2HPO_4	–	5.0	5.0	5.0	–	–
$MgSO_4 \cdot 7H_2O$	–	2.5	2.5	2.5	10	–
$(NH_4)_2SO_4$	–	–	–	–	100	–
Trace metal solution (mL/L)		1	1	1	1	
F1(mL)			300	500		
F2 (mL)			200	200		

A, *B* Media for batch cultures; *C*, *D* Media for fed-batch cultures ; *F1*, *F2* Feeding media

Western blot analysis

One millilitre of cells at $OD_{600} = 1$ was concentrated to 100 μL and mixed with equal amount of 2× sample buffer, boiled for 5 min. Then, 15 μL of the whole cell lysates was loaded to each well and analyzed on 10% SDS-PAGE. Western blot analysis was carried out according to the protocol provided by the supplier (Novagen, Darmstadt, Germany). Protein samples were electroblotted onto PVDF membrane (Perkin Elmer, Boston, MA) and incubated with mouse anti-His monoclonal antibody (Novagen). Subsequently, samples were reacted with secondary goat anti-mouse IgG alkaline phosphatase conjugated antibody

(Novagen). The membrane was then stained with *FAST* BCIP/NBT (Sigma, St. Louis, MO) for visualizing antigen–antibody conjugates.

Transglucosylating activity of *E. coli* displaying transglucosidase

To assay the activity of the recombinant *E. coli* displaying transglucosidase for arbutin synthesis, reaction mixture consisting of 0.08 g lyophilized cells, 100 mM hydroquinone, and 1.2 M maltose in 5 mL of 100 mM sodium phosphate buffer (pH 7.2) was incubated at 40 °C and at 160 rpm for 16 h. Samples were diluted 10 times with deionized water, and the reaction was terminated by heating at 100 °C for 10 min. After heat inactivation, reaction mixture was centrifuged and the supernatant was filtered through a 0.22 μm pore size filter (Millipore, Billerica, MA). The molar conversion was defined as the ratio of the moles of α-arbutin produced to the moles of hydroquinone initially supplied in the reaction mixture under the conditions described above. The enzymatic activity of the whole-cell biocatalyst was defined as follows: one katal (kat) [3] of glucoside-synthesis activity was defined as the amount of enzyme that produced one mole of arbutin per second from hydroquinone after one hour reaction.

Analytical methods

The cell growth was monitored by measuring OD_{600} in a UV/Vis spectrophotometer (DU-530, Beckman, Fullerton, USA), and the dry cell weight was determined by centrifuging 20 mL of the culture in pre-weighed microtubes, washing the pellets with deionized water and drying until constant weight. Arbutin and hydroquinone were quantified by HPLC, using synthetic arbutin (purity ≥98%, Sigma) and *ReagentPlus*®, hydroquinone (purity ≥99%, Sigma) as internal standards. For HPLC analysis, 10 μL of the sample was injected (Autosampler L-2200, Hitachi, Tokyo, Japan) to a LiChrospher RP-18e (4 × 250 mm, Merck, Darmstadt, Germany) column coupled to a UV detector (SPD-6A, Shimadzu, Kyoto, Japan) at 280 nm. The mobile phase consisted of a mixture of methanol and water (3:97 v/v) at 0.7 mL/min. The column was at 35 °C and the retention times for arbutin and hydroquinone under analysis conditions were 8.8 and 10.2 min, respectively. For analyzing glucose, lactose and acetic acid in the culture supernatant by HPLC, 10 μL of sample was injected into an Aminex HPX-87H (7.8 × 300 mm, Bio-Rad, Hercules, CA) column coupled to an RI detector (Bischoff, Leonberg, Germany). The column was at 65 °C and the mobile phase consisted of 5 mM H_2SO_4 at 0.6 mL/min.

Results

Effect of inducer (lactose) concentration

The effect of inducer concentration on fusion protein (INPNC-transglucosidase) expression was investigated in shake-flask cultures. Lactose at concentrations ranging from 0.005 to 60 mM was used and the results are shown in Fig. 1a. The molar conversions of hydroquinone were similar (82%) when lactose at 15–60 mM was used to induce the expression of fusion protein. When the lactose concentration was lowered to 0.1 mM, the molar conversion decreased to 75%. It was found that lactose at 0.005–0.01 mM was not sufficient to induce expression of significant amount of INPNC-transglucosidase and the molar conversion was only 40%. Above findings were further confirmed by the western blot analysis (Fig. 1b). No signals were detected for the fusion protein expression by the cells induced with 0.005–0.01 mM lactose, whereas clear bands corresponding to approximately 90 kDa fusion protein were detected in the western blot analysis for cultures induced with 0.1–60 mM lactose.

Batch culture of recombinant *E. coli*

The batch cultures of recombinant *E. coli* harboring pInaXNC1-aglA were carried out in medium A (Table 1). The cultures grown until OD_{600} = 0.5 were induced once using IPTG at 1 mM. The results are summarized in Table 2 (Ba1), and used as the reference data for further discussion.

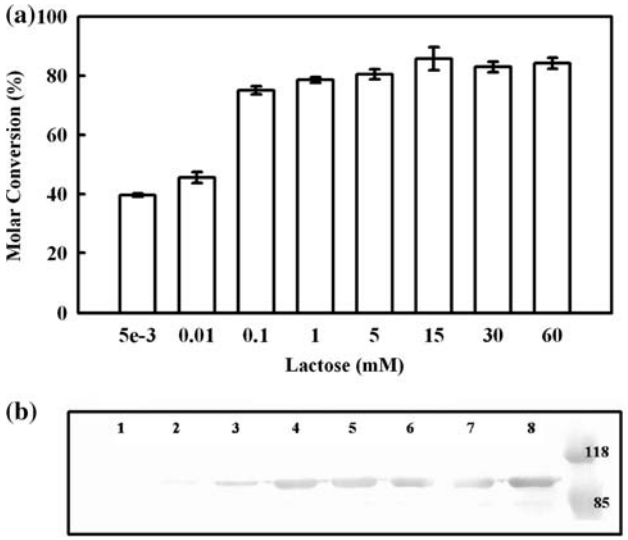

Fig. 1 The effect of lactose concentration on the catalytic activity of recombinant *E. coli* displaying transglucosidase in hydroquinone glucosylation. **a** Molar conversion of α-arbutin by whole cells induced at different lactose concentrations. **b** Western blot analysis of INPNC-transglucosidase fusion protein. Data are mean values ± SD from three independent experiments

Table 2 Summary of fermentation and glucosylation of whole-cell biocatalysts

Mode[a]	Ba1	Ba2	Ba3	Ba4	Fb1	Fb2
Fermentation						
Medium	A	B	B	B	C	D
Temperature shift (°C)	37–25	30–25	30–25	30–25	30–25	30–25
Inducer	IPTG	IPTG	IPTG	Lactose	Lactose	Lactose
Number of induction	1	1	2	1	5	7
Cell mass (g/L)	1.9	3.5	3.2	4.1	13.5	17.6
Productivity (g whole cell biocatalyst/ L h)	0.08	0.13	0.13	0.15	0.40	0.49
Glucosylation						
Specific activity (nkat/g cell)	562.5	495	510	496.2	481.3	501.3
Molar conversion (%)	83.1	74.3	77	74.7	71.7	76.3
Yield of arbutin (g/L)	23	20.2	20.9	20.3	19.5	20.8

[a] *Ba* Batch; *Fb* Fed-batch

To enhance the cell concentration in batch cultures, medium B (containing 2 g/L of glucose as the carbon source) was used. Figure. 2a shows the results of single induction with IPTG at the exponential phase (OD_{600} = 3.2) of growth, Fig. 2b shows the results of inducing twice with IPTG, one at the early exponential phase (OD_{600} = 1) and the other at the late exponential phase (OD_{600} = 3.7) of growth and Fig. 2c shows the results of inducing with lactose when the culture was at OD_{600} = 2.9. The results of these batch cultures are summarized in Table 2 (Ba2, Ba3 and Ba4, respectively). Although the experiments using lactose as inducer resulted in higher biomass than that of others, it seemed that some lactose was used as carbon source when the concentration of glucose in the broth was limited. In all the experiments, glucose was completely exhausted in 6 h after inoculation, and this was considered as the feeding point to initiate the fed-batch culture. Since the transition from batch to fed-batch phase was indicated by the rapid rise in dissolved oxygen, which corresponded to the depletion of the glucose added at the beginning of the batch culture. In addition, the concentration of acetic acid was never higher than that of the inhibition level of growth and protein synthesis (Fig. 2). The concentration of lactose in the culture broth was maintained between 11 and 15 mM after 10 h of induction (Fig. 2c) and corresponded to the value of lactose for an adequate amount of protein expression (Fig. 1).

The transglucosylation activity of the recombinant cells was assayed in hydroquinone glucosylation. The results of the experiments for comparing the effect of the type of inducer are shown in Table 2 (Ba1–Ba4). The specific activities of cells grown under Ba2, Ba3 and Ba4 conditions were slightly lower than that of the reference (Ba1). In the experiments with two pulses of IPTG induction, the specific activity of the recombinant cells was higher (510 nkat/g cell) than those with a single pulse of IPTG or lactose induction. Inducing the culture once with IPTG or lactose, the recombinant cells exhibited very similar specific activities (495 nkat/g cell) for hydroquinone transglucosylation. Therefore, lactose was used instead of the expensive IPTG for expression of INPNC-transglucosidase, since there was no difference in the transglucosylating activities of the whole cell biocatalysts produced by using the two inducers.

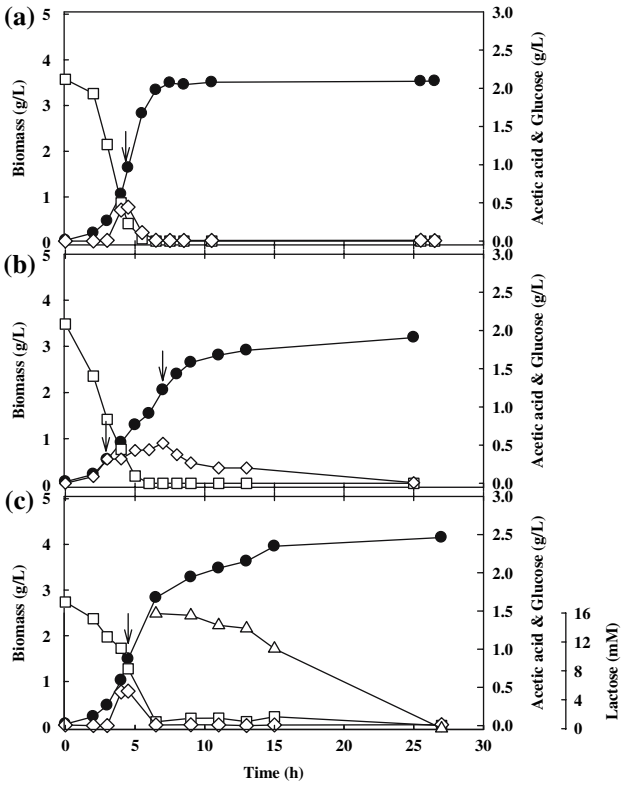

Fig. 2 Biomass (*filled circle*), glucose (*open square*), acetic acid (*open diamond*), and lactose (*open triangle*) concentrations during batch cultures of *E. coli* BL21(λDE3)/pInaXNC1-aglA using **a** one pulse of IPTG, **b** two pulses of IPTG, and **c** one pulse of lactose. *Arrows* indicate feeding of inducers. *The data points* represent the averages of three independent experiments

Fed-batch culture of recombinant *E. coli*

Figure. 3a shows the results of fed-batch culture in medium C with five consecutive pulses of lactose for inducing fusion protein expression. The biomass concentration reached 13.5 g/L, to give an overall biomass yield of 0.3 g/g, based on the total amount of glucose consumed. The concentration of glucose was limited during the entire feeding period and also during induction. On the other hand, the concentration of acetic acid in the culture broth did not exceed 0.5 g/L during the whole cultivation period, which remained below inhibitory levels [22]. The concentration of lactose in the culture broth varied between 5.8 and 18.4 mM during the first 8 h of induction. The lactose concentration in the culture broth declined to 0.8 mM at the final stages of culture.

The whole-cell biocatalysts produced in the fed-batch culture were assayed for the transglucosylating activity in hydroquinone glucosylation and a specific activity of 86% with respect to the reference (Ba1) was obtained. Although the specific activity of cells was slightly lower than that of the reference or the batch culture induced with one pulse of lactose or IPTG, the biomass obtained in this fed-batch culture was 7 times higher than that of the reference (Ba1), and 3–4 times higher than that of batch cultures (Ba2–Ba4; Table 2).

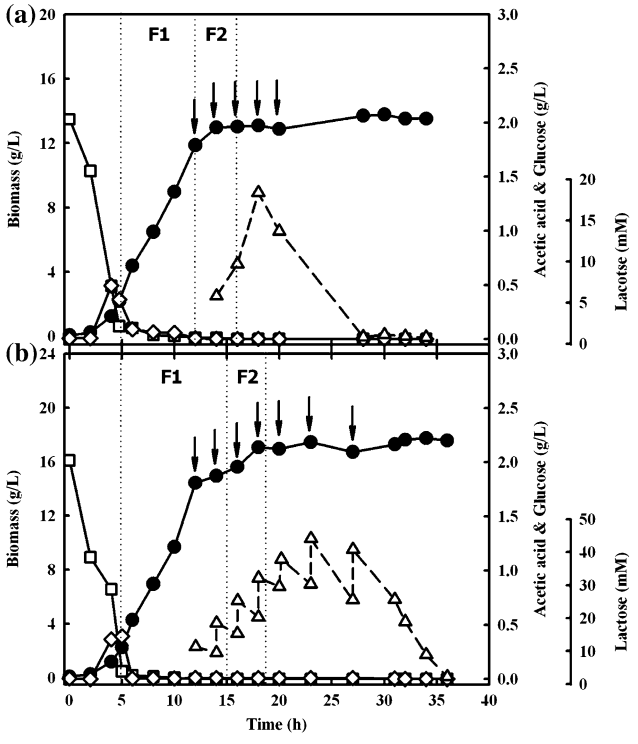

Fig. 3 Biomass (*filled circle*), glucose (*open square*), acetic acid (*open diamond*), and lactose (*open triangle*) concentrations during fed-batch cultures of *E. coli* BL21(λDE3)/pInaXNC1-aglA using **a** five pulses and **b** seven pulses of lactose. *Arrows* indicate feeding of lactose. *F1* indicates feeding of F1 medium and *F2* indicates feeding of F2 medium. *The data points* represent the averages of three independent experiments

The results of fed-batch culture in medium D using seven consecutive pulses of lactose for recombinant protein expression are shown in Fig. 3b. The biomass increased to 17.6 g/L, and an overall biomass yield of 0.26 g/g glucose was obtained after 36 h of cultivation. As in the case of fed-batch culture with five consecutive pulses of lactose, residual glucose in the culture broth remained limited throughout the fed-batch and the induction periods. In addition, acetic acid was never above 0.5 g/L during fed-batch culture. The level of lactose in the culture broth changed between 9 and 44 mM during the first 15 h of induction, and the concentration was maintained above 15 mM between 15 and 32 h of cultivation. The lactose concentration declined to 2 mM in the culture broth at the end of cultivation.

The harvested cells when used as a biocatalyst in hydroquinone glucosylation showed a specific activity of 501 nkat/g cell, and yielded arbutin of 21 g/L. The biomass obtained in this fed-batch culture was 4–6 times higher than that of batch cultures (Ba2–Ba4) and the specific activity of cells was higher than that from the batch culture (Ba4) and from the fed-batch culture using lactose as inducer (Fb1). The productivities of whole cell biocatalyst, and the molar conversions obtained in hydroquinone glucosylation are summarized in Table 2. The fed-batch fermentation with lactose induction resulted in a sixfold increase in the productivity of cells relative to that obtained from the reference batch culture (Ba1).

Discussion

In our previous work on the construction of recombinant *E. coli* displaying transglucosidase, we focused on the enzymatic characteristics of the whole cells in hydroquinone glucosylation [26]. We wish to extend this work to establish a high cell density culture for the production of *E. coli* cells displaying transglucosidase for use as biocatalysts, for large-scale production of α-arbutin by glucosylation of hydroquinone. The fed-batch culture strategy was attempted in this study for the production of large quantities of *E. coli* cells with surface-displayed transglucosidase, and the expression of the fusion protein was induced by using lactose as an inducer.

Glucose is generally used as the carbon source for the high-cell density of *E. coli*. However, the presence of glucose in the cell repressed transcription from the *lac* promoter by reducing the levels of cAMP and influenced the expression of foreign proteins during the induction phase [1, 2]. In fed-batch cultures of *E. coli* displaying

transglucosidase, dissolved oxygen was used as the glucose feed indicator. The level of glucose in the culture broth was never higher than 0.05 g/L after addition of fresh glucose in the two fed-batch cultures using five and seven pulses of lactose (Fig. 3). Since cells with very high catalytic activity were obtained, catabolite repression of T7*lac* promoter for INPNC-transglucosidase expression might not have occurred. Acetate, which was excreted under aerobic conditions that resulted from excess glucose, was another factor that affects both cell growth and recombinant proteins biosynthesis [22]. The concentration of acetic acid never went above 0.5 g/L and remained very low throughout the rest of pre- and post-induction phases in fed batch cultures (Fig. 3). It was well below the inhibitory level of 2 g/L that affected cell growth and recombinant proteins production [22].

The use of IPTG for induction of the *lac* promoter in small-scale cultivations is well established. However, the use of IPTG is strongly limited due to its toxicity towards human being and a strong metabolic burden on the *E. coli* at high concentration which results in low product yields [4]. Lactose is as effective as IPTG for inducing recombinant protein using *lac* promoter and has been discussed in the literatures [2, 6, 7, 12, 17]. In the present study, lactose was found to be a suitable inducer for INPNC-transglucosidase fusion protein, a T7*lac* promoter controlled gene, in the recombinant *E. coli* and as little as 0.1 mM lactose was sufficient to induce the expression of INPNC-transglucosidase (Fig. 1b). However, the molar conversion obtained using whole cells cultivated under 0.1 mM lactose induction was lower than that of the cells cultivated using higher lactose concentrations. For high transglucosylating activity of the whole cells, lactose concentration in the culture broth should not be lower than 5 mM (Fig. 1a). Moreover, these experiments suggested that induction with lactose above 15 mM resulted in the production of whole-cells with the highest transglucosylating activity.

In batch culture using lactose as an inducer, the residual lactose concentration in culture broth was maintained at the value of lactose for an adequate amount of protein expression (Fig. 1) after 10 h induction. The development of appropriate induction strategies including numbers of pulse of lactose addition and time interval between the consecutive pulses should be considered and modified in the fermentor. For further discussion on number of pulses of lactose addition, we performed two runs of fed-batch culture by five and seven pulses of lactose addition at the same time interval. Based on the results presented, lactose in the range of 20–44 mM in the culture broth should be reached for INPNC-transglucosidase production (Fig. 3b), however, these levels of lactose may probably vary with other culture conditions.

The INPNC-transglucosidase is a fusion protein of 90 kDa, and is located on the outer membrane. The fusion proteins, synthesized in the cytoplasm, should be exported to the periplasm and then located on the outer membrane. The lower culture temperatures (25–30 °C) significantly reduced proteolytic degradation, improved the stability of expressed recombinant protein, and reduced protein aggregation, resulting in higher yields of properly folded product [2]. Furthermore, lower temperatures may enhance the proper export, folding and assembly of functional recombinant proteins secreted to the periplasm [2]. For example, for the surface expression of GFP (Lpp-OmpA-GFP), *E. coli* growth at 25 °C resulted in a twofold increase in fluorescence signal above background after 24 h cultivation [20]. Similarly, high activities of surface expressed OPH (Lpp-OmpA-OPH) were observed at 30 °C incubation of *E. coli*, and avoided impairing of the protein folding mechanism, whereas, cells grown at 37 °C led to incorrect insertion of the Lpp-OmpA-OPH fusion into the cell membrane [19]. In shake flask cultures, we have confirmed that when the culture was at 37 °C throughout the growth and the induction phases, the cells did not possess any measurable transglucosylating activity, although the proteins expression level was high on SDS-PAGE (data not shown). When the culture temperature was lowered after inducer addition to 25 °C, the recombinant cells with surface-displayed transglucosidase showed higher activity as compared to incubation at 30 or 37 °C (data not shown). Based on these facts, we suggest that the recombinant *E. coli* (harboring INPNC-transglucosidase) should be grown at 30 °C at the beginning of culture, and then shifted to 25 °C during the induction phase, for obtaining very high transglucosylating activity of recombinant cells.

Conclusions

In this study, we have successfully demonstrated the application of fed-batch culture strategy for the production of recombinant *E. coli* cells anchoring surface-displayed transglucosidase, for use as a biocatalyst in α-arbutin synthesis. Although the transglucosylating activity of recombinant cells using lactose as an inducer was slightly lower than that of the biocatalyst produced by IPTG induction, the use of fed-batch culture by lactose induction resulted in a higher productivity of whole cell biocatalysts. Therefore, fed-batch cultivation coupled with lactose induction offers an attractive strategy for the mass production of recombinant *E. coli* cells for use as whole-cell biocatalysts in biotransformations such as α-arbutin synthesis.

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References

1. Bechwith JR (1987) The lactose operon. In: Neidhardt FC, Ingraham JL, Low KB, Magasanik B, Schaechter M, Umbarger HE (eds) *Escherichia coli* and *Salmonella typhimurium*. Cellular and molecular biology, Vol 2. American Society for Microbiology, Washington, pp 1448
2. Donovan RS, Robinson CW, Glick BR (1996) Optimizing inducer and culture conditions for expression of foreign proteins under the control of the *lac* promoter. J Ind Microbiol 16:145–154
3. Dybkaer R (2001) Unit "katal" for catalytic activity (IUPAC Technol Report). Pure Appl Chem 73:927–913
4. Fu XY, Wei DZ, Tong WY (2006) Effect of yeast extract on the expression of thioredoxin-human parathyroid hormone from recombinant *Escherichia coli*. J Chem Technol Biotechnol 81:1866–1871
5. Funayama M, Arakawa H, Yamamoto R, Nishino T, Shin T, Murao S (1995) Effect of α- and β-arbutin on activity of tyrosinases from mushroom and mouse melanoma. Biosci Biotechol Biochem 59:143–144
6. Gombert AK, Kilikian BV (1998) Recombinant gene expression in *Escherichia coli* cultivation using lactose as inducer. J Biotechnol 60:47–54
7. Hoffman BJ, Broadwater JA, Johnson P, Harper J, Fox BG, Kenealy WR (1995) Lactose fed-batch overexpressioon of recombinant metalloproteins in *Escherichia coli* BL21(DE3): process control yielding high levels of metal-incorporated, soluble protein. Protein Expr Purif 6:646–654
8. Hori I, Nihei KI, Kubo I (2004) Structural criteria for depigmenting mechanism of arbutin. Phytother Res 18:475–479
9. Inomata S, Yokoyama M, Seto S, Yanagi M (1991) High-level production of arbutin from hydroquinone in suspension cultures of *Catharanthus roseus* plant cells. Appl Microbiol Biotechnol 36:315–319
10. Jung HC, Lebeault JM, Pan JG (1998) Surface display of *Zymomonas mobilis* levansucrase by using the ice-nucleation protein of *Pseudomonas syringae*. Nat Biotechnol 16:576–580
11. Jung HC, Park JH, Park SH, Lebeault JM, Pan JG (1998) Expression of carboxymethylcellulase on the surface of *Escherichia coli* using *Pseudomonas syringae* ice nucleation protein. Enzyme Microb Technol 22:348–354
12. Kilikian BV, Suárez ID, Liria CW, Gombert AK (2000) Process strategies to improve heterologous protein production in *Escherichia coli* under lactose or IPTG induction. Process Biochem 35:1019–1025
13. Kitao S, Sekine H (1994) α-D-Glucosyl transfer to phenolic compounds by sucrose phosphorylase from *Leuconostoc mesenteroides* and production of α-arbutin. Biosci Biotechol Biochem 58:38–42
14. Kurosu J, Sato T, Yoshida K, Tsugane T, Shimura S, Kirimura K, Kino K, Usami S (2002) Enzymatic synthesis of α-arbutin by α-anomer-selective glucosylation of hydroquinone using lyophilized cells of *Xanthomonas campestris* WU-9701. J Biosci Bioeng 93:328–330
15. Lutterbach R, Stöckigt J (1992) High-yield formation of arbutin from hydroquinone by cell-suspension cultures of *Rauwolfia serpentine*. Helv Chim Acta 75:2009–2011
16. Maeda K, Fukuda M (1996) Arbutin: mechanism of its depigmenting action in human melanocyte culture. J Pharmacol Exp Ther 276:765–769
17. Neubauer P, Hofmann K, Holst O, Mattiasson B, Kruschke P (1992) Maximizing the expression of a recombinant gene in *Escherichia coli* by manipulation of induction time using lactose as inducer. Appl Microbiol Biotechnol 36:739–744
18. Nishimura T, Kometani T, Takii H, Terada Y, Okada S (1994) Purification and some properties of α-amylase from *Bacillus subtilis* X-23 that glucosylates phenolic compounds such as hydroquinone. J Ferment Bioeng 78:31–36
19. Richins RD, Kaneva I, Mulchandani A, Chen W (1997) Biodegradation of organophosphorus pesticides by surface-expressed organophosphorus hydrolase. Nat Biotechnol 15:984–987
20. Shi H, Su WW (2001) Display of green fluorescent protein on *Escherichia coli* cell surface. Enzyme Microb Technol 28:25–34
21. Shiloach J, Bauer S (1975) High yield growth of *E. coli* at different temperatures in a bench scale fermentor. Biotechnol Bioeng 17:227–239
22. Shiloach J, Fass R (2005) Growing *E. coli* to high cell density—a historical perspective on method development. Biotechnol Adv 23:345–357
23. Shimazu M, Mulchandani A, Chen W (2001) Cell surface display of organophosphorus hydrolase using ice nucleation protein. Biotechnol Prog 17:76–80
24. Sugimoto K, Nishimura T, Nomura K, Sugimoto K, Kuriki T (2003) Syntheses of arbutin-α-glycosides and a comparison of their inhibitory effects with those of α-arbutin and arbutin on human tyrosinase. Chem Pharm Bull 51:798–801
25. Sugimoto K, Nishimura T, Nomura K, Sugimoto K, Kuriki T (2004) Inhibitory effects of α-arbutin on melanin synthesis in cultured human melanoma cells and a three-dimensional human skin model. Biol Pharm Bull 27:510–514
26. Wu PH, Giridhar R, Wu WT (2006) Surface display of transglucosidase on *Escherichia coli* by using the ice nucleation protein of *Xanthomonas campestris* and its application in glucosylation of hydroquinone. Biotechnol Bioeng 95:1138–1147
27. Yokoyama M, Inomata S, Seto S, Yangi M (1990) Effects of sugars on the glucosylation of exogenous hydroquinone by *Catharanthus roseus* cells in suspension culture. Plant Cell Physiol 31:551–555