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Matsuura, Rena ; Kishida, Mayumi ; Konishi, Rie ; Hirata, Yuuki ; Adachi, Noriko ; Segawa, Shota ; Imao, Kenta ; Tanaka, Tsutomu ; Kondo…

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Tsutomu Tanaka ORCID iD: 0000-0002-7397-7360

Akihiko Kondo ORCID iD: 0000-0003-1527-5288

Metabolic engineering to improve 1,5-diaminopentane production from cellobiose using β-glucosidase-secreting *Corynebacterium glutamicum*

Running Title: Improved DAP production using *C. glutamicum*

Rena Matsuura [1], Mayumi Kishida [1], Rie Konishi[1], Yuuki Hirata [1], Noriko Adachi[1], Shota Segawa[1], Kenta Imao[1], Tsutomu Tanaka [1*], Akihiko Kondo [2, 3]

[1]Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University 1-1, Rokkodaicho, Nada, Kobe 657-8501, Japan

[2]Graduate School of Science, Technology and Innovation, Kobe University, 1-1 Rokkodaicho, Nada, Kobe 657-8501, Japan

[3]Center for Sustainable Resource Science, RIKEN, Wako, Saitama 351-0198, Japan.

*Corresponding author: Tsutomu Tanaka

Tel/Fax: +81-78-803-6202

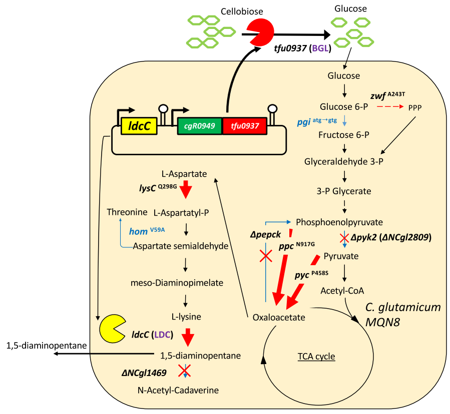
e-mail: tanaka@kitty.kobe-u.ac.jp

Abstract

Microbial production of 1,5-diaminopentane (DAP) from renewable feedstock is a promising and sustainable approach for the production of polyamides. In this study, we constructed a β-glucosidase (BGL)-secreting *Corynebacterium glutamicum* and successfully used this strain to produce DAP from cellobiose and glucose. First, *C. glutamicum* was metabolically engineered to produce L-lysine (a direct precursor of DAP), followed by the co-expression of L-lysine decarboxylase (ldcC) and BGL derived from *Escherichia coli* and *Thermobifida fusca* YX (Tfu0937), respectively. This new engineered *C. glutamicum* strain produced 27 g/L of DAP from cellobiose in CGXII minimal medium using fed-batch cultivation. The yield of DAP was 0.43 g/g glucose (1 g of cellobiose corresponds to 1.1 g of glucose), which is the highest yield reported to date. These results demonstrate the feasibility of DAP production from cellobiose or cellooligosaccharides using an engineered *C. glutamicum* strain.

Keywords; *Corynebacterium glutamicum*; 1,5-diaminopentane; β-glucosidase; cellobiose; metabolic engineering

Graphical abstract



This study demonstrated improved production of 1,5-diaminopentane (DAP) directly from cellobiose using a β-glucosidase (BGL)-secreting *Corynebacterium glutamicum*. This engineered *C. glutamicum* strain produced 27 g/L of DAP from cellobiose in CGXII minimal medium during fed-batch cultivation. The yield of DAP was 0.43 g/g glucose (1 g of cellobiose corresponds to 1.1 g of glucose). These results demonstrate the feasibility of DAP production from cellobiose or cellooligosaccharides using an engineered *C. glutamicum* strain.

1. Introduction

Engineered strains of *Corynebacterium glutamicum* are a standard for the commercial production of L-lysine, with more than 2 million tons of L-lysine per year being produced by fermentation (Eggeling & Bott, 2015). *C. glutamicum* is a nonpathogenic, gram-positive soil bacterium that has been extensively used for the industrial production of various amino acids (Becker et al., 2018; Becker &Wittmann, 2015; Lee et al., 2016; Wendisch et al., 2016), and the demand for amino acids is expected to increase in the future (Eggeling & Bott, 2015). Moreover, *C. glutamicum* has the potential to produce 1,4-butanediamine (putrescine) and 1,5-diaminopentane (DAP; cadaverine) (Buschke et al., 2011, 2013; Kim et al., 2018; Kind et al. 2011, 2014; Kind & Wittmann, 2011; Schneider et al. 2010), organic acids (Chen et al., 2016; Chung et al., 2017; Wieschalka et al., 2013), and aromatic compounds (Kogure et al., 2016; Lee & Wendisch, 2016). Therefore, this bacterium is promising for use in the production of biofuels and commodity chemicals.

DAP is a 5-carbon linear aliphatic diamine and is an industrially relevant platform for the synthesis of polyamides and polyurethanes (Kim et al., 2018; Kind et al., 2011). DAP produced by microbial biosynthesis polymerizes with appropriate organic acids produced by microbial fermentation and enables the production of completely bio-based polyamides with enhanced material properties that are useful in high-value products. Furthermore, polyamides confer properties such as high tensile strength and good elasticity and flexibility to polymers, which can be applied in clinical medicine to produce resin, sutures, and catheters (Jiang & Loos, 2016; Park et al., 2014). DAP is readily produced through the decarboxylation of lysine by lysine decarboxylase (E.C. 4.1.1.18). Bio-based DAP production has attracted considerable attention with regard to microbial engineering for its potential to produce novel bio-based polyamides. The most promising strategy for bio-based DAP production is the use of metabolically engineered microorganisms. DAP production has been demonstrated in *C. glutamicum* (Kim et al., 2018; Kind et al., 2011, 2014; Tateno et al., 2009) and *Escherichia coli* (Ikeda et al., 2013; Qian et al. 2011) through the overexpression of lysine decarboxylases. Moreover, downstream purification processes of fermentation-derived DAP and its polycondensation into a bionylon have been developed (Kim et al., 2018; Kind et al., 2014). Although a broad range of alternative substrates such as starch (Tateno et al., 2009), xylose (Buschke et al., 2011, 2013), and xylooligosaccharides (Imao et al., 2017) have been used in DAP production, cellulosic materials have not yet been used.

The utilization of biomass as a source for fuels and chemicals has attracted much attention for its potential to facilitate the development of sustainable economic growth. Lignocellulosic biomass is regarded as a promising feedstock because this material is abundant, inexpensive, and renewable (Taha et al., 2016). However, most microorganisms cannot directly utilize lignocellulosic biomass, and degradation of this material requires expensive and complex steps. Efficient degradation of cellulose requires a synergistic combination of the cellulolytic enzymes endoglucanase (EG), cellobiohydrolase (CBH), and β-glucosidase (BGL). The cellulose is degraded by EG and CBH to produce cellobiose and some cellooligosaccharides, which can be converted to glucose by BGL. BGL not only catalyzes the final step in cellulose degradation but also stimulates cellulose hydrolysis by alleviating the cellobiose-mediated inhibition of EG and CBH activities (Baumgart et al. 2007). However, commercially available cellulases contain low levels of BGL, which necessitates the addition of large amounts of enzyme to increase saccharification efficiency (Sukumaran et al., 2010). As an initial step toward the direct conversion of lignocellulosic biomass to biofuels and commodity chemicals, and for the reduction of required cellulases, we focused on DAP production from cellobiose using metabolically-engineered *C. glutamicum*.

C. glutamicum has been metabolically engineered to utilize cellobiose by expression of the *bglF317A* and *bglA* genes that respectively encode a variant of the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS)-glucoside-specific enzyme IIBCA component and phospho-BGL (Ikeda, 2012; Sasaki et al., 2008). Furthermore, 4.8 mM L-lysine production has been demonstrated from 20 g/L cellobiose following the expression of BGL (Anusree et al., 2016), which is the rate limiting enzyme in cellulose hydrolysis (Anusree et al., 2016). Alternatively, as demonstrated in our previous work, BGL from *Saccharophagus degradans* (Sde1394) was displayed on the cell surface facilitates direct production of L-lysine from cellobiose (Adachi et al., 2013). This BGL-displaying *C. glutamicum* strain completely consumed 20 g/L of cellobiose in 4 days with a concomitant synthesis of 1.08 g/L (7.4 mM) of L-lysine; however, cellobiose utilization by this strain was considerably slow compared with the glucose consumption of current *C. glutamicum* strains, due to the low enzymatic activity of *S. degradans* BGL. For the efficient DAP production, improvement of BGL activity and L-lysine (i.e. a precursor of DAP) productivity is required.

In the present study, we confronted this issue by constructing novel BGL-expressing *C. glutamicum* strains and re-engineering the metabolic pathways of *C. glutamicum* to improve DAP production. Further, we demonstrated successful DAP production by fed-batch fermentation using cellobiose as the sole carbon source and obtained the highest yield of DAP (0.43 g/g glucose).

2. Materials and methods

2.1 Bacterial strains and media

Bacterial strains and plasmids, including their characteristics and sources, are listed in Table 1. All *C. glutamicum* strains were derived from the wild-type strain ATCC 13032. *C. glutamicum* strains were routinely cultivated aerobically at 30°C in Brain Heart Infusion (BHI) medium (Difco Laboratories, Detroit, USA) or defined CGXII minimal medium (Keilhauer et al., 1993) containing 5% (w/v) glucose or cellobiose as the sole carbon source. *Escherichia coli* NovaBlue, which was used for recombinant DNA experiments, was routinely cultivated in Luria–Bertani medium (10-g/L peptone, 5-g/L yeast extract, and 10-g/L NaCl) at 37°C. Kanamycin (25 µg/mL for *C. glutamicum* strains and 50 µg/mL for *E. coli*) was added when required.

2.2 Construction of plasmids and strains

Detailed descriptions of plasmid and strain construction and primer sequences are summarized in the Supporting Information. Figure 1 illustrates the strains and plasmids constructed in this study.

2.3 Transformation of *C. glutamicum*

C. glutamicum was cultured overnight in 5 mL of BHI medium at 30°C. Further, 1 mL of seed culture was inoculated into 80 mL of BHI medium. After incubation at 30°C until OD600 reached 0.5, the cell suspension was centrifuged at 4,000 × *g* for 5 min and washed three times with 5 mL of 15% (v/v) glycerol before resuspension in 0.5 mL of 15% glycerol. Transformation of *C. glutamicum* was performed by electroporation with a 2.5-kV, 200-Ω, 25-μF electric pulse in a 0.2-cm cuvette using a Gene Pulser (Bio-Rad Laboratories, Hercules, CA, USA).

2.4 Measurement of BGL activity

BGL activity was measured quantitatively in 50-mM sodium acetate buffer (pH 5.0) at 37°C with 1-mM p-nitrophenyl-β-D-xylopyranoside (Nacalai Tesque, Inc., Kyoto, Japan) as the substrate. After overnight cultivation of cells at 30°C in BHI medium, culture supernatants following centrifugation at 4000 × *g* for 10 min were collected and used for BGL measurement. The amount of p-nitrophenol released by BGL was determined by measuring absorbance at 400 nm.

2.5 Cell culture in test-tubes

A single colony was used as the inoculum for preculture (5-ml BHI medium in 35-ml test tubes) and was incubated overnight at 30°C with shaking at 220 rpm. Cells were collected by centrifugation at 4000 × *g* for 5 min and resuspended in 5 mL of 1% NaCl. The suspension (250 μL) was inoculated into 5 mL of CGXII containing 50 g/L cellobiose or 50 g/L glucose and incubated at 30°C with shaking at 220 rpm.

2.6 Fed-batch fermentation

A single colony was precultured in 5 mL BHI medium overnight at 30°C with shaking at 220 rpm. The preculture was inoculated into 100 mL of BHI medium in a 1-L baffled flask and cultured overnight at 30°C with shaking at 180 rpm. Cells were collected by centrifugation at 4000 × *g* for 10 min and inoculated into 400 mL of CGXII medium containing 50 g/L cellobiose in a 1-L jar fermenter. The initial OD600 was adjusted to 0.5 and temperature was maintained at 30°C. The pH was maintained at 7.00 by the automated addition of 28% (v/v) NH_3. Foam formation was suppressed using KM-70 (Shin-Etsu Chemical, Co., Ltd., Tokyo, Japan). Dissolved oxygen (DO) was maintained at >20% by controlling agitation. Fed-batch fermentations were performed until the cellobiose and glucose was consumed. The final volume of the feeding solutions was 200 mL of CGXII containing 100 g/L of cellobiose, and cell growth was monitored by measuring the dry cell weight (DCW). Other metabolites were analyzed as described below.

2.7 Analysis of substrates and products

Glucose and cellobiose concentrations were analyzed using a Prominence high-performance liquid chromatography (HPLC) system (Shimadzu) equipped with a Shodex SUGAR KS-801 column (6 μm, 300 × 8.0 mm). Water was used as the mobile phase at a flow rate of 0.8 mL/min, and the column was maintained at 50°C. Lysine concentrations were analyzed using a Prominence HPLC system equipped with a

COSMOSIL 5 C18-PAQ column (250 × 4.6 mm ID; Nacalai Tesque, Inc.). Twenty mM of phosphate buffer (pH 2.5) containing 10 mM of sodium 1-pentanesulfonate was used as the mobile phase. The flow rate was 1.0 mL/min, and the column was maintained at 30°C. Peak elution profiles were all monitored using a refractive index detector.

3. Results and discussion

3.1 Creation of the L-lysine-producing base strain MQN6 for DAP production

L-lysine is a direct precursor of DAP. Multiple approaches have been used to construct strains for L-lysine production, including metabolic engineering to improve titer, conversion rate, and yield. As an initial step toward DAP production, we created an L-lysine-producing *C. glutamicum* strain, designated MQN6, in which negative feedback by L-lysine and L-aspartate was eliminated by the introduction of *lysC*^{Q298G} (Chen et al., 2011) and *ppc*^{N917G} (Chen et al., 2013) mutations, respectively. Threonine formation was decreased by the introduction of a *hom*^{V58A} mutation in homoserine dehydrogenase, whereas the anaplerotic reaction was improved by the deletion of the gene encoding PEP carboxykinase and introduction of a variant (*pyc*^{P458S}) of the pyruvate carboxylase gene. Furthermore, the strain was partially cured of prophage (Bott et al. 2013). Based on the accumulated knowledge regarding L-lysine production (Heider & Wendisch, 2015; Becker et al., 2011; Wendisch, 2014), several additional mutations (including *zwf*^{A243T} and a mutation that converted the *pgi* start codon from ATG to GTG, a lesion known to enhance NADPH availability) were introduced (Becker et al., 2011). The engineered strains, which are summarized in Figure 1 and Table 1, were used in subsequent experiments.

To convert L-lysine into DAP, *ldcC* that encodes lysine decarboxylase in *E. coli* was overexpressed under synthetic promoters, H30 and H36 (Yim et al., 2013). Both promoters were strong and constitutive. Figure 2 shows DAP production from 50 g/L glucose as the sole carbon source. The MQN6-6L strain produced 13 g/L of DAP after 48 h, and MQN6-0L produced 8.1 g/L of DAP after 72 h; however, the growth and glucose consumption were similar in both strains. Kim et al. (2018) achieved high DAP production (12.5 g/L from 50g/L of glucose) using a *C. glutamicum* PKC strain under the control of the H30 promoter, which was found to be superior to the H36 promoter in the study (9.7 g/L of DAP). Oh et al. (2015) reported no expression of ldcC under the control of the H36 promoter using *C. glutamicum* KCTC1857 as a host.

These observations suggest that the suitability of a promoter for *ldcC* expression is dependent on the host cell being used (Becker et al., 2018). MQN6 was derived from *C. glutamicum* ATCC 13032, which favors the H36 promoter for *ldcC* expression (Fig. 2) and is in agreement with the original report (Yim et al., 2013). L-lysine was not detected in the culture medium, demonstrating sufficient ldcC activity under the control of the H36 promoter.

3.2 Construction of BGL-secreting strains and DAP production using cellobiose as the sole carbon source

We previously reported the construction of a *C. glutamicum* strain that displayed BGL from *S. degradans* on its surface (Adachi et al., 2013). Notably, the cellobiose consumption rate of this strain was considerably low, even during growth in a nutrient-rich complex medium. One major challenge was to improve this low BGL activity; therefore, we redesigned the BGL expression system in *C. glutamicum*. In the present study, we used BGL Tfu0937 from *Thermobifida fusca* YX, which has been shown to exhibit high BGL activity in *E. coli* (Tanaka et al., 2011), and the signal sequence of CgR0949 from *C. glutamicum* R (Teramoto et al., 2011, Yim et al., 2016) to construct a Tfu0937-secreting strain. The CgR0949 signal peptide drives Tat-dependent secretion, which is known to be employed by other classes of cellulases such as xylanase (Yim et al., 2016). BGL was expressed as a fusion of the CgR0949 signal sequence to a codon-optimized Tfu0937. The plasmids designed for co-expression of *BGL* and *ldcC* are shown in Figure 1B, and H30 and H36 promoters were used for *BGL* and *ldcC* expression, respectively. Figure 3A shows the cell growth of the resulting MQN6 strains harboring the *BGL* and *ldcC* co-expression plasmids during culture in CGXII medium that contained 50 g/L of cellobiose as the sole carbon source. The OD600 of two strains with BGL under the control of the H36 promoter approached 9.8 and 7.1 after 24 h; furthermore, BGL activity was detected and increased in the culture media, suggesting successful BGL secretion (Fig. 3B). The strains with the H30 promoter controlling BGL expression and the control strain failed to grow on cellobiose, and BGL activity was not detectable in the media (data not shown). BGL expression under the control of H30 promoter was confirmed by SDS-PAGE analysis (data not shown), suggesting lower secretion efficiency might cause some stress and the growth failure on cellobiose.

Figure 4 shows DAP production from glucose as the sole carbon source by the MQN6-6L6B and MQN6-0L6B strains. MQN6-6L6B accumulated 11.5 g/L of DAP after 40 h of cultivation, whereas MQN6-0L6B accumulated 5.5 g/L of DAP after 72 h. The growth rate of MQN6-6L6B (0.269 h^{-1}) cells was higher than that of MQN6-0L6B (0.263 h^{-1}) despite their similar ability to consume glucose. These results clearly demonstrate the superiority of the H36 promoter with regard to ldcC and BGL expression. The amount of DAP produced by MQN6-6L and MQN6-6L6B was 13 g/L (Fig. 2) and 11.5 g/L (Fig. 4), respectively. High amount of L-lysine is toxic and may causes cell death (Stabler et al., 2011; Kim et al., 2018), however, L-lysine was not detected in the culture medium. It implying that the slight decrease in DAP production was caused by a little stress of BGL expression. The titer of L-lysine produced from 50 g/L cellobiose using the MQN6 strain with only the BGL expression was 9.7g/L (data not shown), which was significantly improved compared to our previous report (1.08 g/L; Adachi et al., 2013). It also suggests that the conversion of L-lysine to DAP increase of the L-lysine branch flux.

When cellobiose was used as the sole carbon source, MQN6-6L6B produced 13 g/L of DAP after 72h from 50 g/L of cellobiose (Fig. 5). This corresponds to a yield of 0.26 g DAP/g glucose (1 g of cellobiose corresponds to 1.1 g of glucose), which is slightly higher than that obtained from glucose (0.23 g DAP/g glucose; Fig. 2). Previously reported yields of DAP from glucose using batch cultivation (Table 2) are 0.23 (Kind et al., 2014) and 0.25 g/g glucose (Kim et al., 2018), which are similar to our results from cellobiose. Cell growth on cellobiose was slightly slower than that on glucose, and cellobiose was completely hydrolyzed by 42 h. However, free glucose was detected in the culture medium between 24 h and 48 h, indicating that BGL-based conversion of cellobiose into glucose was sufficient and that glucose uptake requires improvement to enhance DAP production.

3.3 Enhancement of glucose uptake by deletion of *iolR* or flux rearrangement by deletion of *pyk* genes

Free glucose generated by BGL was detected in the culture medium (Fig. 5), indicating that the secreted form of BGL provided sufficient activity for growth by cellobiose hydrolysis. Therefore, engineering of this strain to enhance glucose uptake may improve DAP production. Accordingly, we deleted the transcriptional regulator-encoding gene *iolR* in *C. glutamicum*, which reportedly results in the activation of genes involved in inositol metabolism (Klaffl et al., 2013) and enhanced glucose uptake through a non-PTS route (Ikeda et al., 2011; Lindner et al., 2011). However, as shown in Figure 6, *iolR* deletion in the MQN6 strain (which produced strain MQN7), resulted in decreased DAP production (7.4 g/L) from 50 g/L of cellobiose (Fig. 6; blue symbols), whereas glucose and cellobiose consumption by MQN7-6L6B did not improve and was similar to that by MQN6-6L6B. Thus, another approach was used to improve DAP production.

To improve DAP production, we attempted to direct metabolic flux toward the production of oxaloacetate, a precursor of L-lysine and DAP, and focused on pyruvate kinase, pyk, an enzyme that converts phoshoenolpyruvate into pyruvate. This enzyme is important with regard to the control of intermediate metabolism; however, the effects of its deletion on L-lysine production (a direct precursor of DAP) depends on the genetic background of the strain (Becker et al., 2018). Positive effects on L-lysine production using pyk-defective variants have been reported (Siio et al., 1987, 1990), whereas negative effects have been reported in other pyk-deletion strains (Becker et al., 2008; Gubler et al., 1994; Park et al., 1997). However, Yanase et al. (2016) have demonstrated the potential of pyk deletion in combination with a feedback inhibition-resistant phosphenolpyruvate carboxylase (PPC) variant (D299N) for enhancing the anaplerotic reaction with improved lysine production in *C. glutamicum* (Yanase et al., 2016). Here, we deleted *pyk2* (cg3218, NCgl2809) and *pyk* (cg2291, NCgl2008) in the MQN6 strain. The *pyk2* gene codes for pyruvate kinase (Chai et al., 2016). The catalytic activity of Pyk2 was allosterically regulated by fructose 1,6-bisphosphate (FBP) activation and ATP inhibition. The mRNA level of pyk2 under oxygen deprivation was higher than that under aerobic conditions (Chai et al., 2016). However, the effect of its deletion on DAP production has not been reported. Further, we examined DAP production by the resulting *pyk*-deletion strains harboring the ldcC and BGL co-expression plasmid, i.e., MQN8-6L6B (*pyk2* deletion) and MQN9-6L6B (*pyk* deletion). Cell growth and sugar consumption were similar in MQN9-6L6B MQN6-6L6B (Figure 6A, C); however, *pyk* deletion significantly decreased DAP production to 3.7 g/L from 50 g/L of cellobiose (Fig. 6B; yellow symbols). Interestingly, *pyk2* deletion slightly improved DAP production from 13 g/L (MQN6-6L6B; Fig. 5) to 14 g/L (MQN8-6L6B; Fig. 6). The titer of L-lysine produced from 50 g/L cellobiose using the MQN8 strain with only the BGL expression was 12.9 g/L (data not shown), which was higher than that of MQN-6 strain (9.7 g/L; data now shown). Chai et al. reported that pyk2 and ldhA (L-lactate dehydrogenase) were cotranscribed as a bicistronic mRNA under aerobic condition. In the case of MQN6-6L6B and MQN8-6L6B, both strains had same levels of ldh activity in the crude extract (data not shown), suggesting less effect of pyk2 deletion on ldh activity. Cell growth of MQN8-6L6B was slightly slower than other strains, possibly because of *pyk2* deletion. Moreover, *pyk* deletion causes a rearrangement of the fluxes through anaplerotic reactions, such as a flux shift from pyruvate carboxylase to PPC (Becker et al., 2008). Thus, feedback-resistant PPC is required (Yanase et al., 2016) for enhanced L-lysine production. Our results demonstrated that *pyk2* deletion improved DAP production because of an enhanced L-lysine supply.

3.4 Fed-batch cultivation using cellobiose as the sole carbon source

We evaluated DAP production under fed-batch conditions with the limited cellobiose feeding using CGXII medium supplemented with cellobiose as the sole carbon source. The cells hydrolyzed cellobiose into glucose approaching a high cell concentration (30 g/L of DCW) during initial batch cultivation. Free glucose generated by BGL from cellobiose digestion was detected in the culture medium, suggesting the presence of sufficient BGL activity. During the batch phase, 17.5 g/L of DAP was produced with a yield of 0.37 g /g glucose (1 g of cellobiose corresponds to 1.1 g of glucose), which is higher than that of test-tube cultivation (0.26 g-DAP/g-glucose; Fig. 6). This improved yield may have resulted from improved culture conditions such as oxygen supply and pH control. Further cellobiose feeding was initiated after 52 h of cultivation, when most of the original cellobiose and residual glucose was exhausted. Under these conditions, MQN8-6L6B ultimately produced 27 g/L of DAP after 96 h of cultivation (Fig. 7A), corresponding to a yield of 0.43 g DAP/g glucose. In addition, the yield was 0.49 g DAP/g glucose during the feeding phase; however, the cell concentration decreased slightly toward the end of the cultivation (Fig. 7B). In contrast to the cellobiose-supplied batch phase, free glucose was not detected during the feeding phase (Fig. 7B), whereas levels of several by-products, such as lactate, acetate, pyruvate, succinate, and glycerol, remained below 0.5 g/L throughout the cultivation. L-lysine, a precursor of DAP, was not detected in the medium. Notably, the yield reported here, i.e., 0.46 g DAP/g glucose (1 g cellobiose is equivalent to 1.1 g glucose), is substantially superior to those previously reported for DAP production using *C. glutamicum* (Table 2).

4. Conclusion

The *C. glutamicum* strain MQN8-6L6B was metabolically engineered for enhanced DAP production from cellobiose by optimizing its metabolic pathway and expressing highly active BGL. Fed-batch fermentation of MQN8-6L6B, which expresses *E. coli* lysine decarboxylase and secretes *T. fusca* BGL, produced 27 g/L of DAP from cellobiose, which represents the highest yield of DAP reported to date. This study represents an important step in developing an economically feasible and sustainable process for DAP production when combined with downstream processes such as purification and polymerization (Kind et al., 2014, Kim et al, 2018).

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review.

Conflicts of interest

The authors declare no conflicts of interest.

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Figure legends

Figure 1. Metabolic engineering of *Corynebacterium glutamicum* for 1,5-diaminopentane (DAP) production from cellobiose (A) and constructs of β-glucosidase and L-lysine decarboxylase co-expression (B). Abbreviations: BGL, β-glucosidase; *pgi*, glucose-6-phosphate isomerase; *pyk*, pyruvate kinase; *pepck*, phosphoenolpyruvate carboxykinase; *ppc*, phosphoenolpyruvate carboxylase; *pyc*, pyruvate carboxylase; *lysC*, aspartokinase; *hom*, homoserine dehydrogenase; *ldcC*, L-lysine decarboxylase; *zwf*, glucose-6-phosphate dehydrogenase.

Figure 2. 1,5-Diaminopentane (DAP) production using β-glucosidase-secreting strain MQN6-0L and MQN6-6L. CGXII medium containing 50 g/L of glucose as the sole carbon source was used. Cell growth (A), 1,5-diaminopentane concentrations (B), and glucose concentrations (C) are shown. Blue symbols indicate MQN6-0L and red symbols indicate MQN6-6L.

Figure 3. Growth on cellobiose as the sole carbon source using β-glucosidase-secreting *Corynebacterium glutamicum* under the control of H36 and H30 promoter. O.D. (A) and time profiles of β-glucosidase activity in culture supernatant (B) are shown. Blue symbols indicate MQN6-0L6B and red symbols indicate MQN6-6L6B. Data are presented as mean ± standard deviation calculated from the results of three independent experiments. Abbreviations: BGL, β-glucosidase.

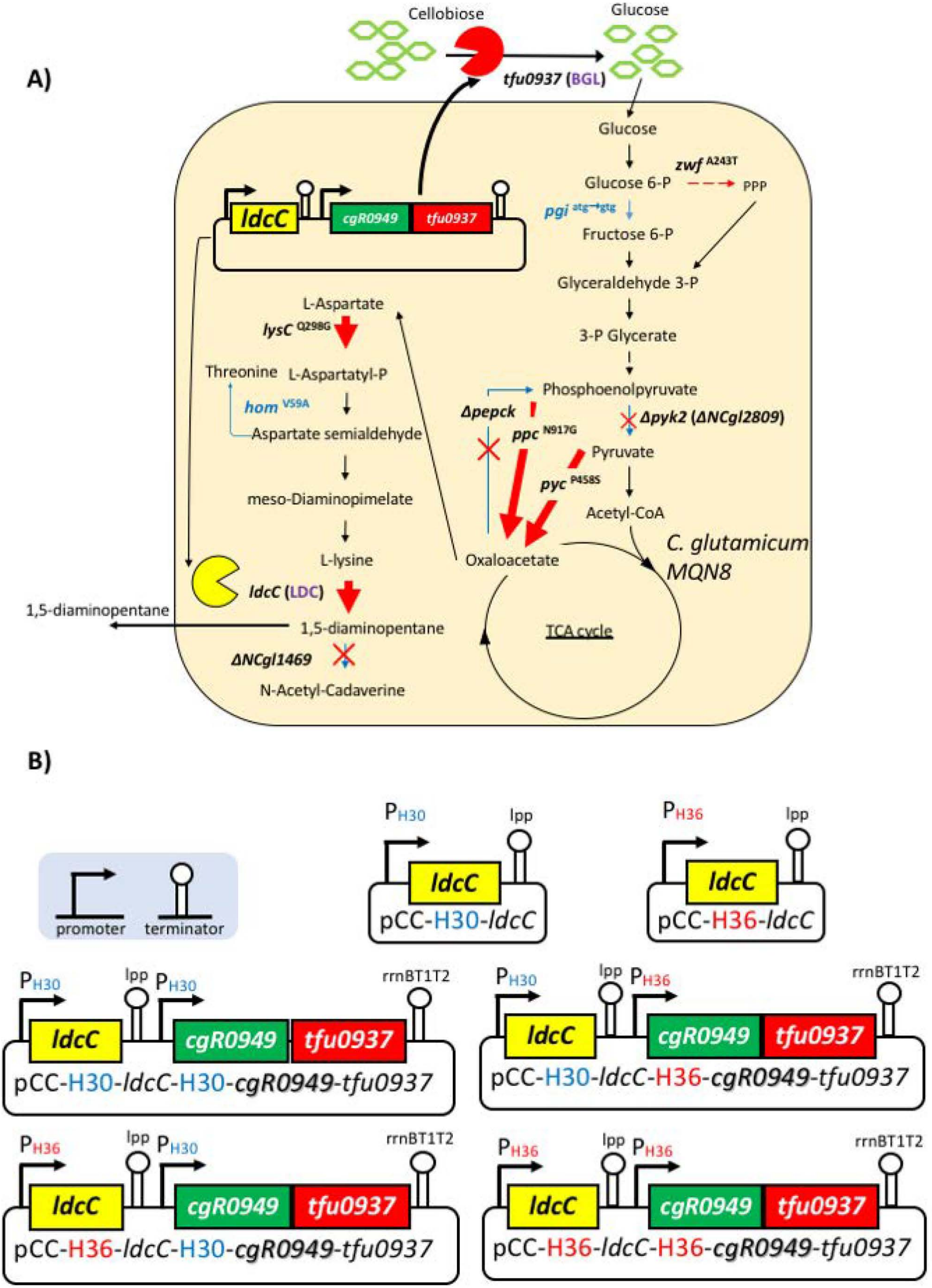
Figure 4. 1,5-Diaminopentane (DAP) production by the MQN6-0L6B and MQN6-6L6B strains from 50 g/L of glucose in CGXII medium. Cell growth (A), 1,5-diaminopentane concentrations (B), and glucose concentrations (C) are shown. Blue symbols indicate MQN6-0L6B and red symbols indicate MQN6-6L6B. Data are presented as mean ± standard deviation calculated from the results of three independent experiments.

Figure 5. 1,5-Diaminopentane (DAP) production by the MQN6-6L6B strains from 50 g/L of cellobiose in CGXII medium. Cell growth (A), 1,5-diaminopentane concentrations (B), and sugar concentrations (C) are shown. Free glucose detected in the culture medium during cultivation is indicated by open circles with dotted line. Data are presented as mean ± standard deviation calculated from the results of three independent experiments.

Figure 6. 1,5-Diaminopentane (DAP) production by the MQN7-6L6B, MQN8-6L6B, MQN9-6L6B strains from 50 g/L of cellobiose in CGXII medium. Cell growth (A), 1,5-diaminopentane concentrations (B), and sugar concentrations (C) are shown. Blue square symbols indicate MQN7-6L6B, red diamond symbols indicate MQN8-6L6B and yellow circle symbols indicate MQN9-6L6B. Free glucose detected in the culture medium during cultivation is indicated by open squares and diamonds with dotted lines. Data are presented as mean ± standard deviation calculated from the results of three

independent experiments.

Figure 7. Fed-batch cultivation of strain MQN8-6L6B using cellobiose as the sole carbon source. Time courses of 1,5-diaminopentane (A), sugars and dry cell weight (DCW) (B) are shown. The concentration of 1,5-diaminopentane (red circles), of cellobiose (green triangles), of glucose generated from cellobiose by β-glucosidase (dotted green squares), and DCW (blue circles) are shown. Data are presented as mean ± standard deviation calculated from the results of three independent experiments.


A)
Cellobiose
Glucose
tfu0937 (BGL)
ldcC
cgR0949
tfu0937
Glucose
Glucose 6-P
zwf A243T
PPP
pgi atg→gtg
Fructose 6-P
Glyceraldehyde 3-P
3-P Glycerate
Phosphoenolpyruvate
Δpepck
Δpyk2 (ΔNCgl2809)
ppc N917G
Pyruvate
pyc P458S
Acetyl-CoA
Oxaloacetate
TCA cycle
C. glutamicum MQN8
L-Aspartate
lysC Q298G
Threonine
L-Aspartatyl-P
hom V59A
Aspartate semialdehyde
meso-Diaminopimelate
L-lysine
ldcC (LDC)
1,5-diaminopentane
1,5-diaminopentane
ΔNCgl1469
N-Acetyl-Cadaverine
B)
promoter
terminator
PH30
lpp
ldcC
pCC-H30-ldcC
PH36
lpp
ldcC
pCC-H36-ldcC
PH30
lpp
PH30
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PH36
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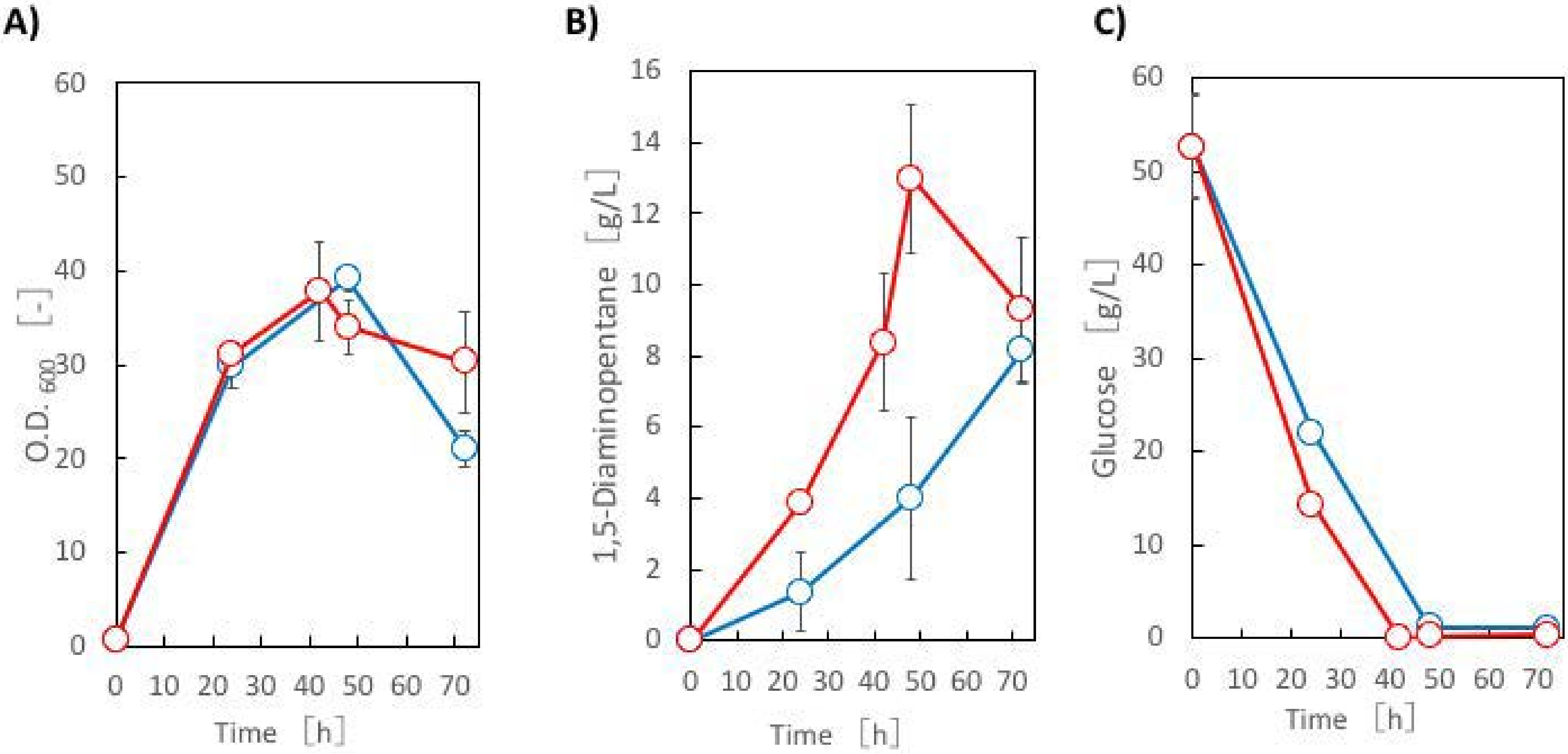
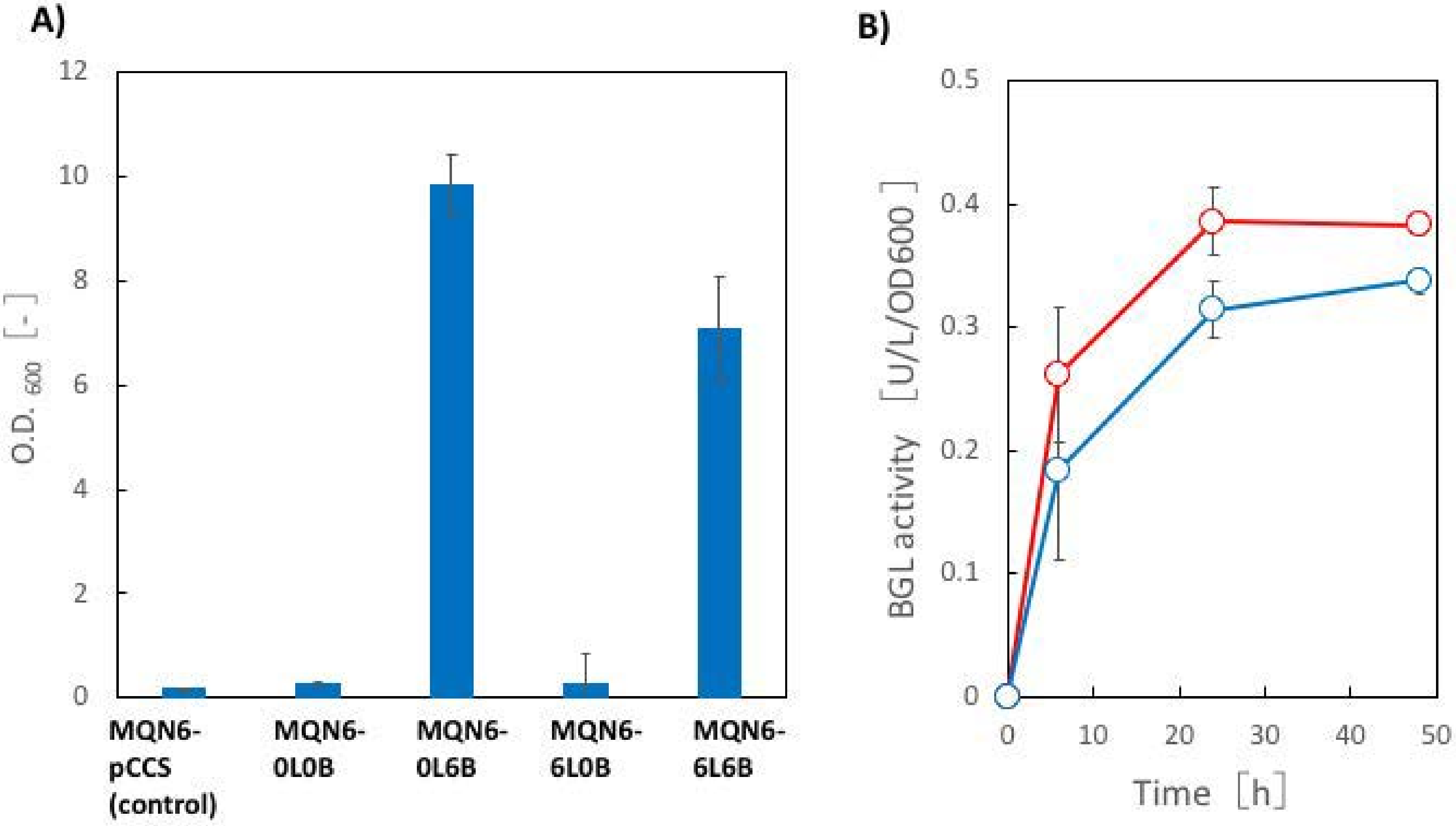
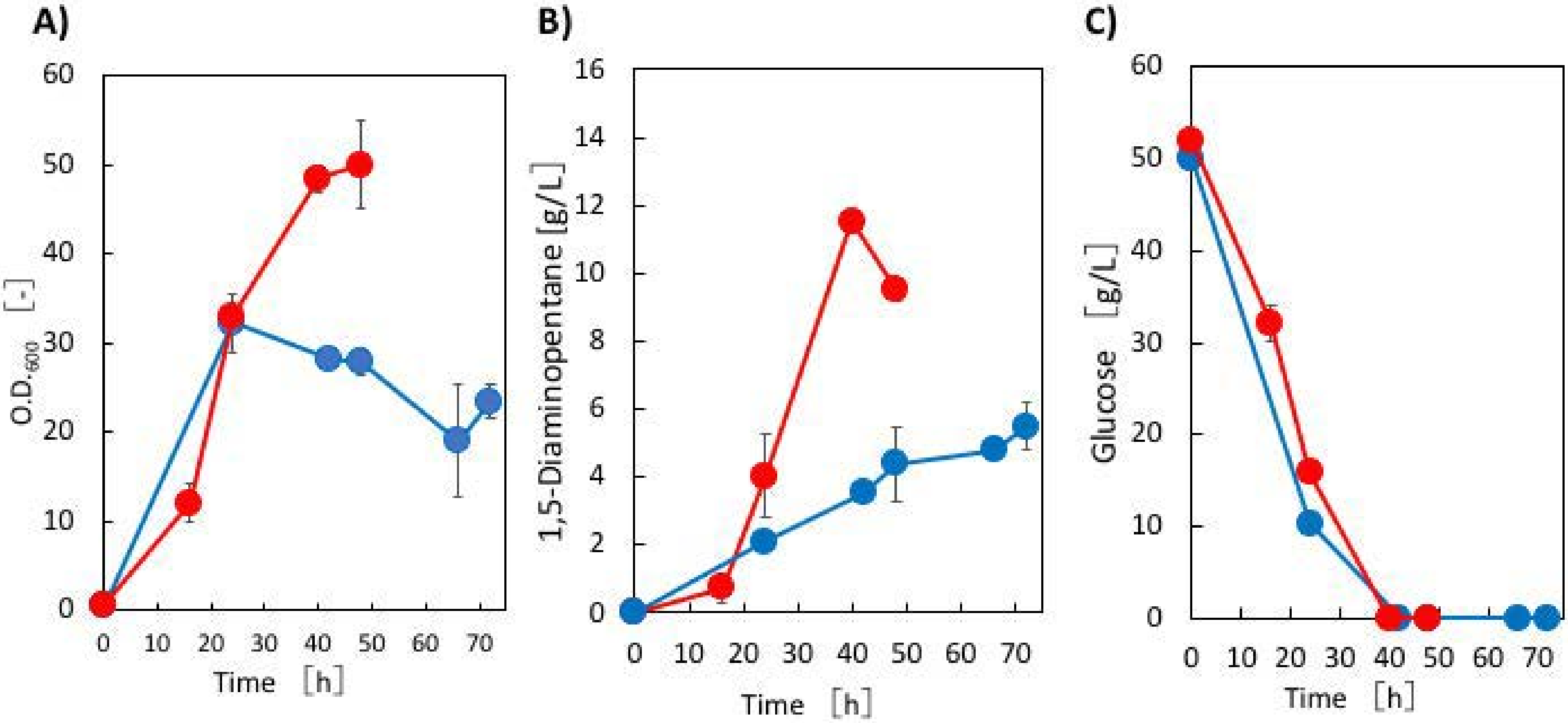
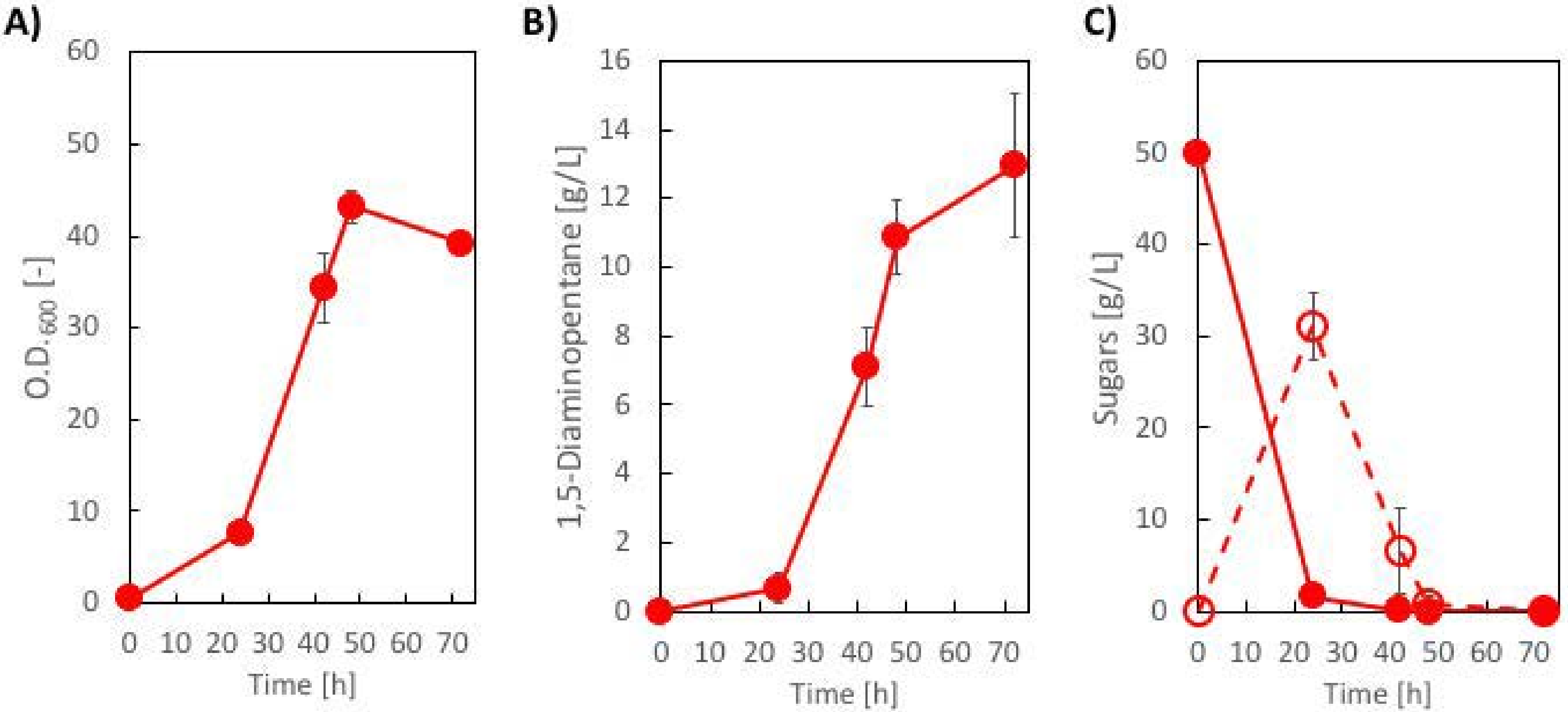
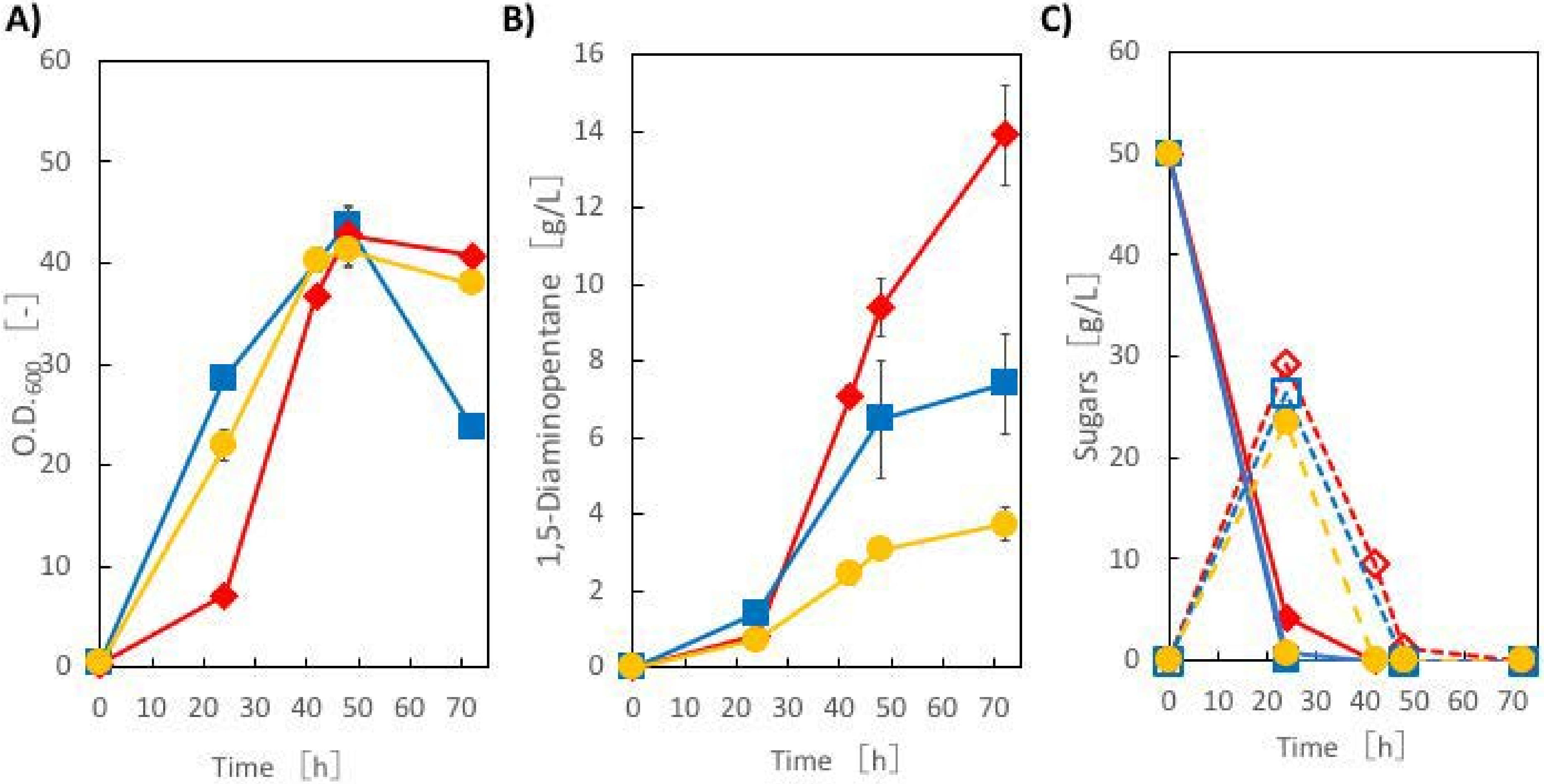
ldcC
cgR0949
tfu0937
pCC-H30-ldcC-H36-cgR0949-tfu0937
PH36
lpp
PH30
rrnBT1T2
ldcC
cgR0949
tfu0937
pCC-H36-ldcC-H30-cgR0949-tfu0937
PH36
lpp
PH36
rrnBT1T2
ldcC
cgR0949
tfu0937
pCC-H36-ldcC-H36-cgR0949-tfu0937

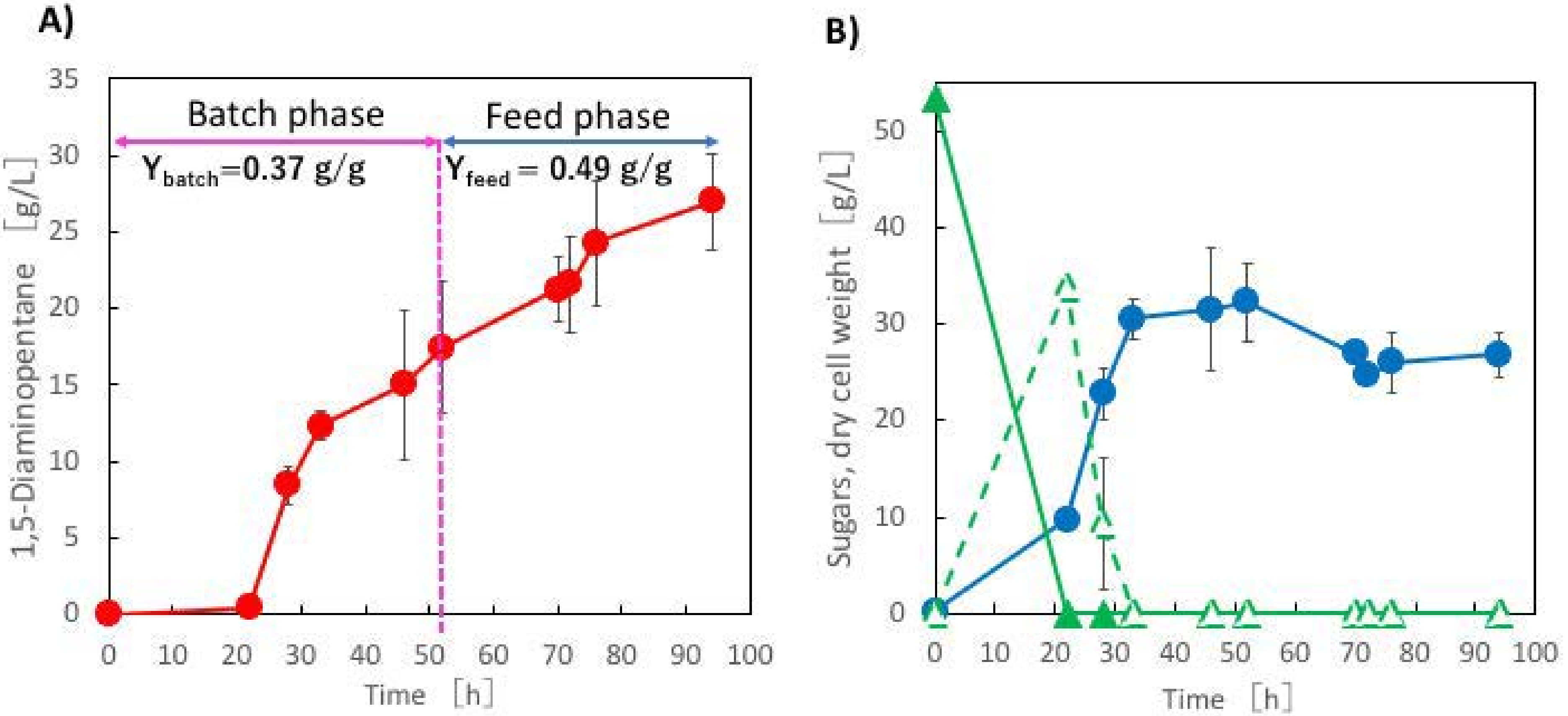

A)
O.D. $_{600}$ [-]
0
10
20
30
40
50
60
0 10 20 30 40 50 60 70
Time [h]
B)
1,5-Diaminopentane [g/L]
0
2
4
6
8
10
12
14
16
0 10 20 30 40 50 60 70
Time [h]
C)
Glucose [g/L]
0
10
20
30
40
50
60
0 10 20 30 40 50 60 70
Time [h]


A)
O.D. 600 [-]
0
2
4
6
8
10
12
MQN6-pCCS (control)
MQN6-0L0B
MQN6-0L6B
MQN6-6L0B
MQN6-6L6B
B)
BGL activity [U/L/OD600]
0
0.1
0.2
0.3
0.4
0.5
0
10
20
30
40
50
Time [h]

A)
B)
C)
O.D.600 [-]
1,5-Diaminopentane [g/L]
Glucose [g/L]
Time [h]
Time [h]
Time [h]
0
10
20
30
40
50
60
0
2
4
6
8
10
12
14
16
0
10
20
30
40
50
60
70


A)
O.D.$_{600}$ [-]
0
10
20
30
40
50
60
Time [h]
0 10 20 30 40 50 60 70
B)
1,5-Diaminopentane [g/L]
0
2
4
6
8
10
12
14
16
Time [h]
0 10 20 30 40 50 60 70
C)
Sugars [g/L]
0
10
20
30
40
50
60
Time [h]
0 10 20 30 40 50 60 70


A)
O.D.$_{600}$ [-]
0
10
20
30
40
50
60
Time [h]
0
10
20
30
40
50
60
70
B)
1,5-Diaminopentane [g/L]
0
2
4
6
8
10
12
14
16
Time [h]
0
10
20
30
40
50
60
70
C)
Sugars [g/L]
0
10
20
30
40
50
60
Time [h]
0
10
20
30
40
50
60
70


A)
Batch phase
Feed phase
Y_batch=0.37 g/g
Y_feed = 0.49 g/g
1,5-Diaminopentane [g/L]
0
5
10
15
20
25
30
35
Time [h]
0
10
20
30
40
50
60
70
80
90
100
B)
Sugars, dry cell weight [g/L]
0
10
20
30
40
50
Time [h]
0
10
20
30
40
50
60
70
80
90
100

Tables

Table1. Strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Reference or source
Strains		
Escherichia coli		
NovaBlue	*endA*1, *hsdR*17, (r$_K$- m$_K$+), *supE*44, *thi*-1, *gyrA*96, *relA*1, *lac, recA*1/F', [*proAB*+, *lacI*qZΔM15, Tn*10* (Tet^R)]	Novagen
SCS110	*rpsL (Str1) thr leu endA thi-l lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB)* [F' *traD36 proAB lacIqZΔM15*]	STRATAGENE

Corynebacterium glutamicum		
ATCC13032	Wild-type strain	ATCC
MQN6	ATCC13032 +*lysC*^{Q298G}(Aspartokinase); ΔcglMRR (cg1996-cg1998); Δ*pepck* (PEP-carboxykinase); Δ*NCgl1469* (N-acetyltransferase); *pyc*^{P485S} (pyruvate carboxylase); *hom*^{V59A} (homoserine dehydrogenase); *ppc*^{N917G} (phosphoenolpyruvate carboxykinase),; *zwf*^{A1243T} (glucose-6-phosphate dehydrogenase); *pgi* atg→gtg start codon (glucose-6-phosphate isomerase)	This study
MQN7	MQN7 *ΔiolR*	This study
MQN8	MQN7 *Δpyk2 (Ncgl2809)*	This study
MQN9	MQN7 *Δpyk (Ncgl2008)*	This study

MQN6-0L	MQN6 harboring pCC-H30-ldcC	This study
MQN6-6L	MQN6 harboring pCC-H36-ldcC	This study
MQN6-0L0B	MQN6 harboring pCC-H30-ldcC-H30-cgR0949-Tfu0937	This study
MQN6-0L6B	MQN6 harboring pCC-H30-ldcC-H36-cgR0949-Tfu0937	This study
MQN6-6L0B	MQN6 harboring pCC-H36-ldcC-H30-cgR0949-Tfu0937	This study
MQN6-6L6B	MQN6 harboring pCC-H36-ldcC-H36-cgR0949-Tfu0937	This study
MQN7-6L6B	MQN7 harboring pCC-H36-ldcC-H36-cgR0949-Tfu0937	
MQN8-6L6B	MQN8 harboring pCC-H36-ldcC-H36-cgR0949-Tfu0937	
MQN9-6L6B	MQN9 harboring pCC-H36-ldcC-H36-cgR0949-Tfu0937	

Plasmids		
pCCS	Vector encoding PorH anchor protein and harboring MCS, Km^R	Tateno et al. 2007
pCC-H30-cgR0949-Tfu0937	pCCS derivative carrying sequences encoding the CgR0949 secretion signal fused to *T. fusca* Tfu0937 under the control of the H30 promoter	This study
pCC-H36-cgR0949-Tfu0937	pCCS derivative carrying sequences encoding the CgR0949 secretion signal fused to *T. fusca* Tfu0937 under the control of the H36 promoter	This study
pCC-H30-ldcC	pCCS derivative carrying sequences encoding the ldcC from *E. coli*, under the control of the H30 promoter	This study

pCC-H36-ldcC	pCCS derivative carrying sequences encoding the ldcC from *E. coli*, under the control of the H36 promoter	This study
pCC-H30-ldcC-H30-cgR0949-Tfu0937	pCCS derivative carrying sequences encoding the ldcC from *E. coli*, under the control of the H30 promoter; CgR0949 secretion signal, Tfu0937 from *T. fusca*, under the control of the H30 promoter;	This study
pCC-H30-ldcC-H36-cgR0949-Tfu0937	pCCS derivative carrying sequences encoding the ldcC from *E. coli*, under the control of the H30 promoter; CgR0949 secretion signal, Tfu0937 from *T. fusca*, under the control of the H36 promoter;	This study

pCC-H36-ldcC-H30-cgR0949-Tfu0937	pCCS derivative carrying sequences encoding the ldcC from *E. coli*, under the control of the H36 promoter; CgR0949 secretion signal, Tfu0937 from *T. fusca*, under the control of the H30 promoter;	This study
pCC-H36-ldcC-H36-cgR0949-Tfu0937	pCCS derivative carrying sequences encoding the ldcC from *E. coli*, under the control of the H36 promoter; CgR0949 secretion signal, Tfu0937 from *T. fusca*, under the control of the H36 promoter;	This study
pK18mobsacB	sacB, lacZ, Km^R, MCS, mobilizable vector, enables selection/counter-selection for integration/excision in *C. glutamicum*	ATCC
pK18mobsacB-$lysC^{Q298G}$	pK18mobsacB derivative for substitution of $lysC^{Q298G}$	This study

pK18mobsacB-pyc^{P485S}	pK18mobsacB derivative for substitution of *pyc*^{P485S}	This study
pK18mobsacB-hom^{V59A}	pK18mobsacB derivative for substitution of *hom*^{V59A}	This study
pK18mobsacB-ppc^{N917G}	pK18mobsacB derivative for substitution of *ppc*^{N917G}	This study
pK18mobsacB-zwf^{A243T}	pK18mobsacB derivative for substitution of *zwf (A243T)*	This study
pK18mobsacB-pgi$^{atg\text{-}gtg}$	pK18mobsacB derivative for substitution of *pgi* with start codon ATG→GTG	This study
pK18mobsacB-ΔcglMRR	pK18mobsacB derivative for *cglMRR* deletion	This study
pK18mobsacB-Δpepck	pK18mobsacB derivative for *pepck* deletion	This study
pK18mobsacB-ΔNcgl1469	pK18mobsacB derivative for *Ncgl1469* deletion	This study
pK18mobsacB-ΔiolR	pK18mobsacB derivative for *iolR* deletion	This study
pK18mobsacB-Δpyk	pK18mobsacB derivative for *pyk* deletion	This study

pK18mobsacB-Δpyk2	pK18mobsacB derivative for *pyk2* deletion	This study

Table 2. 1,5-Diaminopentane production reported previously.

Substrate	Condition	Strain	1,5-diaminopentane titer (g/L)	Yield (g 1,5-diaminopentane/g glucose)	Reference
Glucose (50 g/L)	Batch / test-tube	*C. glutamicum* MQN6-6L6B	11.5	0.23	This study
Cellobiose (50 g/L)	Batch / test-tube	*C. glutamicum* MQ86-6L6B	13.9	0.26	This study
Cellobiose (57 g/L)	Fed-batch / Jar Fermenter	*C. glutamicum* MQN8-6L6B	27.0	0.43	This study
Glucose	Batch	*C. glutamicum*	1.79	0.23	Kind et al.,

(7.7 g/L)	/ flask	DAP-16			2014
Glucose (310 g/L)	Fed-batch / Jar Fermenter	*C. glutamicum* DAP-16	88	0.28	Kind et al., 2014
Glucose (50 g/L)	Batch / flask	*C. glutamicum* G-H30	12.5	0.25	Kim et al., 2018
Glucose (342 g/L)	Fed-batch / Jar Fermenter	*C. glutamicum* G-H30	103.8	0.30	Kim et al., 2018

Supplementary Materials

Construction of plasmids and strains

All DNA oligonucleotides used in this study are listed in Table S1.

Gene deletion or substitution plasmids were constructed as follows. The *Corynebacterium glutamicum lysC* gene was amplified from strain ATCC 13032 by polymerase chain reaction (PCR) using the primer pair NdeI_LysC_for and XhoI_LysC_re. The resulting fragment was digested using NdeI and XhoI and ligated into similarly digested pET22b (Novagen).

A point mutation was introduced into the cloned *lysC* gene using a KOD-Plus-Mutagenesis kit (TOYOBO, Osaka, Japan) with the primer pair QC_Q298G_for and QC_Q298G_re. The $lysC^{Q298G}$ fragment was amplified by PCR using the primer pair EcoRI_LysC_Q298G_for and BamHI_LysC_Q298G_re, and the resulting fragment was digested using EcoRI and BamHI and ligated into similarly digested pK18mobsacB (ATCC number: 87097). The resulting plasmid was designated pK18mobsacB-lysC$^{\mathrm{Q298G}}$. Other plasmids used for the introduction of point mutations (zwf^{A243T}, hom^{V58A}, and pyc^{P458S}) in *C. glutamicum* genes were constructed in a similar manner.

The plasmid pK18mobsacB-ppc$^{\mathrm{N917G}}$ was constructed as follows. The *C. glutamicum* gene encoding PEP carboxylase (ppc^{N917G}) was amplified from strain ATCC 13032 by PCR using the primer pair EcoRI_ppc_for and BamHI_ppcN917_re. the resulting fragment was digested using EcoRI and BamHI and ligated into similarly digested pK18mobsacB.

The plasmid for the *pepck* gene deletion was constructed as follows. The upstream and downstream regions of the *C. glutamicum pepck* gene were amplified from strain ATCC 13032 by PCR using the primer pairs EcoRI_pepck_Up_for and pepck_Up_BamHI_re and PstI_pepck_down_for and pepck_down_Hind3_re, respectively. The amplified fragments were digested using EcoRI, BamHI or PstI with HindIII (respectively) and ligated into similarly digested pK18mobsacB. The plasmid for the *Ncgl1469* gene deletion was constructed in a similar manner.

With regard to the *cglMRR* deletion, the upstream and downstream regions were amplified from strain ATCC 13032 by PCR using the primer pairs EcoRI_cglMRR_UP_for and cglMRR_UP_re and cglMRR_down_for and BamHI_cglMRR_down_re, respectively. The two fragments were conjugated by overlap PCR using the primer pair EcoRI_cglMRR_UP_for and BamHI_cglMRR_down_re, and the resulting fragment was digested using EcoRI and BamHI and ligated into similarly digested pK18mobsacB. Other plasmids for gene deletion (*iolR*, *pyk, and pyk2*) and minor start codon insertion for *pgi* were constructed in a similar manner.

All mutations of the *C. glutamicum* genome were introduced by homologous recombination and two-step selection, using kanamycin resistance to select for plasmid integration and the *sacB* system to counterselect for plasmid excision.

The plasmid for the expression of secreted β-glucosidase (BGL) was constructed as follows. Sequences encoding the *C. glutamicum* CgR0949 secretion signal sequence fused to a codon-optimized BGL from *T. fusca* (purchased from Life Technologies) were amplified from the BGL-encoding sequence by PCR using the primer pair BamHI_sscgR0949_op0937_for_1 and opTfu0937_XhoI_re. The resulting fragment was subjected to a second round of PCR using the primer pair BamHI_sscgR0949_for_2 and opTfu0937_XhoI_re. Further, the resulting fragment was subjected to a third round of PCR using the primers H36_BamHI_sscgR0949_for and opTfu0937_XhoI_re, and the resulting fragment was digested using BamHI and XhoI. Simultaneously, the plasmid pCC-PorC-Sde1394 (Adachi et al., 2013) was amplified by PCR using the primer pair pCC_H36_PorC_for and pCC_H36_re, and the resulting fragment was self-ligated and then digested using BamHI and XhoI. Next, these two BamHI/XhoI-ended fragments were ligated together to generate a plasmid designated pCH36-cgR0949-Tfu0937 that encodes BGL with an N-terminal secretion signal peptide. The plasmid pCH30-cgR0949-Tfu0937 for expression of secreted BGL under the control of the H30 promoter was constructed in a similar manner using the primer pair H30_BamHI_sscgR0949_for and pCC_H30_re.

The plasmid for the co-expression of lysine decarboxylase (ldcC) and BGL was constructed as follows. Sequences encoding the codon-optimized ldcC from *E. coli* (purchased from Life Technologies) were amplified by PCR using the primer pair H36_BamHI_ldcC_for and ldcC_tlpp_XhoI_re_1. The resulting fragment was subjected to a second round of PCR using the primer pair H36_BamHI_ldcC_for and tlpp_XhoI_2_re. The resulting fragment was ligated with BamHI/XhoI digested pCH36-cgR0949-Tfu0937 to produce a plasmid designated pCC-H36-ldcC.

To construct a plasmid for the co-expression of ldcC and BGL, the fragment encoding the H36 promoter and BGL was amplified by PCR using the primer pair XhoI_tpp_H36_for and opTfu0937_XhoI_re. The resulting fragment was ligated with XhoI-digested pCC-H36-ldcC to generate pCC-H36-ldcC-H36- cgR0949-Tfu0937. Other plasmids were constructed in a similar manner.

Table S1. Primers used in this study

EcoRI_cglMRR_UP_for	ACATGATTACGAATTCATCTCAAGCGACCGTTCAAAAGC
cglMRR_UP_re	CCCATCCACTAAACTTAAACAACAATAGTGGGTTTTGTACTCATG
cglMRR_down_for	TGTTTAAGTTTAGTGGATGGGCATACAAACACCGCTGTTGATTAC
BamHI_cglMRR_down_re	TCGACTCTAGAGGATCCTAAGCTCGCTGACATGCGGTTG
EcoRI_pgi_for	ACATGATTACGAATTCGATTCCCTGGCTGTTCA
pgiGTG_re	TGTCCGCCACGCCCCCTCCT
pgiGTG_for	AGGAGGGGGCGTGGCGGACA
BamHI_pgi_re	TCGACTCTAGAGGATCCGAGCCACCGATACCAATG

EcoRI_iolR_UP_for	ACATGATTACGAATTCGAGGTACTTGCCGAAAGATTG
BamHI_iolR_down_re	CGACTCTAGAGGATCCATCGCGTTGGCATTCTTC
iolR_UP_re	CTCGATTACTTGGCCGGAGGGCTACTTGGAAGTAGAGG
iolR_down_for	TCCGGCCAAGTAATCGAG
EcoRI_pyk_UP_for	ACATGATTACGAATTCGTCTGAGCTGATCCTACCGATCGCTGTG
BamHI_pyk_down_re	CGACTCTAGAGGATCCGATGCGCTTGGAAGTGAAGTCCTCTTCC
pyk_UP_re	GATCACGCAGTTCTGGCCTTTCAACAAGAGACCGCCAAGGGTG
pyk_down_for	GGTCTCTTGTTGAAAGGCCAGAACTGCGTGATCGCATTTGTGC
EcoRI_pyk2_UP_for	ACATGATTACGAATTCCATCGTCGGCGATGTCATGGACAGC
BamHI_ pyk2_down_re	CGACTCTAGAGGATCCCAATAAAGAGGCTTAGAAGCAATTCTGGAGCCTC

pyk2_down_for	GAGTGTTTGTAGCTTAAGGAGCTCAATAACTCACAAAGGCGATTGGCGTTAACTTCGAG
pyk2_UP_re	CGCCTTTGTGAGTTATTGAGCTCCTTAAGCTACAAACACTCTAAATTCCATCC
EcoRI_Ncgl1649_Up_for	ACATGATTACGAATTCCCATGTGCGCATCCTGGGG
Ncgl1649_Up_XhoI_BamHI_re	CGACTCTAGAGGATCCCTCGAGAGAGGCAACCCCATACCAATGTCC
PstI_ Ncgl1649_down_for	CCTCTAGAGTCGACCTGCAGAAGCAGTATGCAGTGGCGGG
Ncgl1649_down_Hind3_re	GGCCAGTGCCAAGCTTGTTCGACAAAGCCTAATAAGGCACC
EcoRI_pepck_Up_for	ACATGATTACGAATTCCATTACTTTAAGCCTTTGGGGCAGTG
pepck _Up_XhoI_BamHI_re	CGACTCTAGAGGATCCCTCGAGCCCCCTAGCGTAGCTTTCAGATACAG
PstI_ pepck _down_for	CCTCTAGAGTCGACCTGCAGTGCAGCTCACTGACTCCGAG
pepck _down_Hind3_re	GGCCAGTGCCAAGCTTGTCTGGGTAACCAGTGGGACG

NdeI_LysC_for	GAAGGAGATATACATATGGCCCTGGTCGTACAGAAATA
XhoI_LysC_re	TGGTGGTGGTGCTCGAGGCGTCCGGTGCCTGCATAAA
QC_Q298G_for	GATGCAGAAATCAACATTGACATGGTTCTGGGGAACGTCTCTTCTGTAGAAGACGGCACCACC
QC_Q298G_re	GGTGGTGCCGTCTTCTACAGAAGAGACGTTCCCCAGAACCATGTCAATGTTGATTTCTGCATC
EcoRI_LysC_Q298G_for	GAGCCTGAATTCGCCCTGGTCGTACAGAAATA
BamHI_LysC_Q298G_re	ATTAATGGATCCGCGTCCGGTGCCTGCATAAA
In_EcoRI_ppc_for	ACATGATTACGAATTCATGACTGATTTTTTACGCGATGACATC
BamHI_ppcN917_re	TCGACTCTAGAGGATCCCTAGCCGGAGCCGCGCAGCGCAGTGGAAAG

NdeI_zwf_for	GAAGGAGATATACATATGGTGAGCACAAACACGACCCC
XhoI_zwf_re	TGGTGGTGGTGCTCGAGTTATGGCCTGCGCCAGGTG
QC_A243T_for	CGTTGACCACGTCCAGATCACCATGactGAAGATATTGGCTTGGGTGGACGTGC
QC_A243T_re	GCACGTCCACCCAAGCCAATATCTTCagtCATGGTGATCTGGACGTGGTCAACG
EcoRI_zwf_A243T_for	GAGCCTGAATTCGTGAGCACAAACACGACCCC
BamHI_zwf_A243T_re	ATTAATGGATCCTTATGGCCTGCGCCAGGTG
NdeI_f-hom_for	GAAGGAGATATACATATGGAGGGGAACTTGATCAGAGGAATACACC
In_XhoI_hom_re	TGGTGGTGGTGCTCGAGTTAGTCCCTTTCGAGGCGGATC
QC_hom_V58A_for	GGCCCACTGGAGGTTCGTGGCATTGCTgctTCTGATATCTCAAAGCCACGTGAAGGCGTTGCACCTG

QC_hom_V58A_re	CAGGTGCAACGCCTTCACGTGGCTTTGAGATATCAGAagcAGCAATGCCACGAACCTCCAGTGGGCC
EcoRI_ hom_V58A _for	ACATGATTACGAATTCGAGGGGAACTTGATCAGAGGAATACACC
BamHI_ hom_V58A _re	TCGACTCTAGAGGATCCTTAGTCCCTTTCGAGGCGGATC
NdeI_pyc_for	GAAGGAGATATACATATGGTGTCGACTCACACATCTTCAACGC
XhoI_pyc_re	TGGTGGTGGTGCTCGAGTTAGGAAACGACGACGATCAAGTC
QC_pyc_P458S_for	CGCCACCGGATTCATTGCCGATCACTCGCACCTCCTTCAGGCTCCACCTGCTGATGATG
QC_pyc_P458S_re	CGCCACCGGATTCATTGCCGATCACTCGCACCTCCTTCAGGCTCCACCTGCTGATGATG
EcoRI_ pyc_P458S _for	ACATGATTACGAATTCGTGTCGACTCACACATCTTCAACGC
BamHI_ pyc_P458S _re	TCGACTCTAGAGGATCCTTAGGAAACGACGACGATCAAGTC

pCC_H36_re	GACCACCCTGGGCCCGTTAATATTCCCCCGTTTAGGGCACCAGATAGAGAATTCCTCGACCAACAGTTGCGC
pCC_H36_PorC_for	CGGGCCCAGGGTGGTCGCACCTTGGTTGGTAGGAGTAGCATGGGATCCATGAAGAAACTACGTTTCGCCACC
pCC_H30_re	GCCACGGGCAACACCACGAATGCGCTACCTTAACCGAAAAGTTACTTTGAATTCCTCGACCAACAGTTGCGC
pCC_H30_PorC_for	GGTGTTGCCCGTGGCCCGGTTGGTTGGGCAGGAGTATATTGGGATCCATGATGAAGAAACTACGTTTCGCCACC
BamHI_sscgR0949_op0937_for_1	CAGGACTTGCCACTATCGGCGCTGCCAGCATGTTTATGCCAAAGGCCAACGCCCTTGGAGCATCTACCTCTCAGTCTACCACCCCGC

opTfu0937_XhoI_re	ACAGCCAAGCCTCGAGCTATTCCTGACCGAAAATACCGCCG
BamHI_sscgR0949_for_2	CGCCTCTATGGGATCCCAAATAAACCGCCGAGGCTTCTTAAAAGCCACCGCAGGACTTGCCACTATCGGCGCTGCCAGCATG
H36_BamHI_sscgR0949_for	GAGTAGCATGGGATCCATGCAAATAAACCGCCGAGGCTTCTTAAAAG
H30_BamHI_sscgR0949_for	GAGTATATTGGGATCCATGCAAATAAACCGCCGAGGCTTCTTAAAAG
H30_BamHI_ldcC_for	GAGTATATTGGGATCCATGAACATTATCGCTATTATGGGTCCTCAC
H36_BamHI_ldcC_for	GAGTAGCATGGGATCCATGAACATTATCGCTATTATGGGTCCTCAC
ldcC_tlpp_XhoI_re_1	GTCGCACAATGTGCGCCATTTTTCACTTCACAGGTTTACTAGCCTGCCATCTTCAGAACACGAAC
tlpp_XhoI_2_re	ACAGCCAAGCCTCGAGGTAGCGGTAAACGGCAGACAAAAAAAATGTCGCACAATGTGCGCC

XhoI_tpp_H30_for	TTACCGCTACCTCGAGAAAGTAACTTTTCGGTTAAGGTAGCGC
XhoI_tpp_H36_for	TTACCGCTACCTCGAGTCTATCTGGTGCCCTAAACGGGGG