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PII: S0168-1656(16)31632-7
DOI: http://dx.doi.org/doi:10.1016/j.jbiotec.2016.11.033
Reference: BIOTEC 7737

To appear in: *Journal of Biotechnology*

Received date: 19-9-2016
Revised date: 30-11-2016
Accepted date: 30-11-2016

Please cite this article as: Chin, Young-Wook, Kim, Ji-Yeong, Kim, Jae-Han, Jung, Sang-Min, Seo, Jin-Ho, Improved production of 2′-fucosyllactose in engineered Escherichia coli by expressing putative α-1,2-fucosyltransferase, WcfB from Bacteroides fragilis.Journal of Biotechnology http://dx.doi.org/10.1016/j.jbiotec.2016.11.033

Improved production of 2'-fucosyllactose in engineered *Escherichia coli* by expressing putative α-1,2-fucosyltransferase, WcfB from *Bacteroides fragilis*

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Research highlights

- 2'-Fucosyllactose (2-FL) is a value-added human milk oligosaccharide in terms of nutraceutical and pharmaceutical purpose.
- The *wcfB* gene coding for α-1,2-fucosyltransferase from *Bacteroides fragilis* was screened from eleven candidates of putative α-1,2-fucosyltransferase.
- The replacement of FucT2 from *Helicobacter pylori* with WcfB from *B. fragilis* leads to improvement of 2-FL titer by 10 fold in batch fermentation of engineered *E. coli*.
- 2-FL concentration, yield and productivity were enhanced further by 50%, 62% and 100% through the complete deletion of *lacZ.*
- Engineered *E. coli* deleting *lacZ* and expressing GDP-L-fucose biosynthetic enzymes and WcfB produced 15.4 g/L extracellular 2-FL from glycerol and lactose in fed-batch fermentation.

Abstract

2'-Fucosyllactose (2-FL) is one of most abundant oligosaccharides in human milk, which is involved in many biological functions for infant health. Since 2-FL has a great potential in application to functional food materials and pharmaceuticals, several microbial systems for mass production of 2-FL have been developed in recent years. Microbial production of 2-FL was suggested to be influenced by a number of factors including fucosylation activity of α-1,2-fucosyltransferase. In the present study, the *wcfB* gene coding for α-1,2-fucosyltransferase from *Bacteroides fragilis* was screened from eleven candidates of putative α-1,2-fucosyltransferase. Introduction of the *wcfB* gene allows the *lacZ*-deleted strain of *E. coli* expressing the genes for guanosine 5'-

diphosphate (GDP)-L-fucose biosynthetic enzymes to produce 2-FL. As a result of fed-batch fermentation, 15.4 g/L extracellular concentration of 2-FL with 2-FL yield of 0.858 g/g lactose and productivity of 0.530 g/L/h were obtained. In addition, the feasibility of industrial production of 2-FL using this microbial system was demonstrated by performing fed-batch fermentation in a 75 L bioreactor.

Keywords: 2'-fucosyllactose; engineered *Escherichia coli*; α-1,2-fucosyltransferase; WcfB

1. Introduction

2'-Fucosyllactose (2-FL) is one of the most abundant oligosaccharides in human milk (Castanys-Muñoz et al., 2013). In addition to the nutritional importance in infant care, the nutraceutical and pharmaceutical potential of 2-FL necessitates the mass production through chemical or biological processes (Chin et al., 2013; Han et al., 2012). Among the factors governing the microbial production of 2-FL, the activity of α-1,2-fucosyltransferase is crucial which catalyzes the final synthesis of 2-FL from GDP-L-fucose and lactose (Fig. 1). α-1,2-Fucosyltransferase, belonging to the glycosyltransferase family 11 (http://afmb.cnrsmrs.fr/CAZY/fam/acc_GT.html) can be found in both eukaryotic and prokaryotic organisms. It transfers the L-fucose molecule from GDP-L-fucose to the galactose moiety of acceptor molecules such as *N*-acetyllactosamine, lacto-*N*-biose and lactose residues observed in oligosaccharides, glycoproteins, or glycolipids (Ma et al., 2006; Oriol et al., 1999).

Several putative bacterial α-1,2-fucosyltransferases have been identified to date, including enzymes involved in colanic acid synthesis in *E. coli* K-12, *Salmonella*

enterica LT2, in O-antigen synthesis in *Yersinia enterocolitica* O8 (Reeves et al., 2006), the WbsJ in the enteropathogenic *E. coli* O128 strain (Li et al., 2008; Shao et al., 2003), the WbgL in the enteropathogenic *E. coli* O126 (Engels and Elling, 2014), WbnK and WbwK of the pathogenic *E. coli* O86 (Yi et al., 2005) as well as WbiQ from *E. coli* O127 (Pettit et al., 2010) and FucT2 (or FutC) from *Helicobacter pylori* (Wang et al., 1999; Wang et al., 2002). Due to the vast knowledge of its functionality and the characteristics, α-1,2-fucosyltransferase from *H. pylori* (FucT2) has been widely used for enzymatic- and microbial production of 2-FL (Albermann et al., 2001; Baumgärtner et al., 2013; Drouillard et al., 2006; Petschacher and Nidetzky, 2016). However, the low level of soluble FucT2 expression as well as its activity in recombinant *E. coli* clearly hinder the production of 2-FL, consequently reduce the overall productivity (Lee et al., 2012; Wang et al., 1999).

In our previous research, several attempts have been performed to improve the 2-FL production. To redirect the lactose flux from cell growth to 2-FL production, the endogenous *lac* operon in the host *E. coli* BL21star(DE3) was replaced with the modified *lac* operon bearing *lacZ⊿M15* which alleviates the β-galactosidase activity (Chin et al., 2015). FucT2 from *H. pylori* was engineered as well to enhance the solubility and activity by attachment of three aspartate molecules at the N-terminal of FucT2. As results, 2-FL production was increased up to 6.4 g/L in the fed-batch fermentation of the engineered *E. coli* (Chin et al., 2015), however, the 2-FL titer was still insufficient to produce 2-FL industrially.

In this study, α-1,2-fucosyltransferases from various origins have been evaluated to overcome the critical point for the 2-FL production by the engineered *E. coli* expressing the genes for the *de novo* biosynthesis of GDP-L-fucose. In addition, β-

galactosidase activity of the host *E. coli* strain was completely eliminated to enhance 2-FL production further. Finally, fed-batch fermentation was carried out in a laboratory scale as well as a pilot scale bioreactor to assess the feasibility of industrial production of 2-FL.

2. Materials and methods

2.1. Strains and plasmids

All *E. coli* strains and plasmids used in this study are listed in Table 1. *E. coli* TOP10 and *E. coli* BL21star(DE3) (Invitrogen, Carlsbad, CA, USA) were used for construction of plasmids and a host strain for production of 2-FL, respectively. Plasmid pBCGW was constructed previously for overexpression of the genes encoding GDP-L-fucose biosynthetic enzymes (ManB, ManC, Gmd and WcaG) (Lee et al., 2009).

The eleven candidate genes coding for α-1,2-fucosyltransferases were selected by comparing amino acid sequences with FucT2 from *H. pylori* at the carbohydrate-active enzymes database (CAZy) site. The DNA fragments encoding α-1,2-fucosyltransferases were amplified by PCR from the genomic DNAs of the appropriate sources (Table 1). The PCR products were digested with *Nde*I and *Kpn*I and ligated into plasmid pCOLADuet-1 digested by the same restriction enzymes. All constructs were verified by restriction enzyme digestion and DNA sequencing.

2.2. Culture conditions

Batch fermentations were done in a 500 mL baffled flask (Nalgene) containing

100 mL of defined medium [13.5 g/L KH_2PO_4, 4.0 g/L $(NH_4)_2HPO_4$, 1.7 g/L citric acid, 1.4 g/L $MgSO_4 \cdot 7H_2O$, 10 ml/L trace element solution (10 g/L Fe(III) citrate, 2.25 g/L $ZnSO_4 \cdot 7H_2O$, 1.0 g/L $CuSO_4 \cdot 5H_2O$, 0.35 g/L $MnSO_4 \cdot H_2O$, 0.23 g/L $Na_2B_4O_7 \cdot 10H_2O$, 0.11 g/L $(NH_4)_6Mo_7O_{24}$, 2.0 g/L $CaCl_2 \cdot 2H_2O$), pH 6.8] with appropriate antibiotics (ampicillin 50 μg/mL and kanamycin 50 μg/mL) at 25°C. The agitation speed was maintained at 250 rpm. When optical density (OD_{600}) reached 0.8, isopropyl-*β*-D-thiogalactopyranoside (IPTG) and lactose were added at a final concentration of 0.1 mM and 20 g/L, respectively. Fed-batch fermentation was carried out in a 2.5 L bioreactor (Kobiotech, Incheon, Korea) containing 1.0 L of defined medium containing 20 g/L glycerol and appropriate antibiotics at 25°C. After complete utilization of glycerol added initially, feeding solution containing 800 g/L glycerol and 20 g/L $MgSO_4 \cdot 7H_2O$ was fed by a pH-stat mode. At the same time, IPTG and lactose were also added to a final concentration of 0.1 mM and 20 g/L for induction of the T7 promoter-mediated gene expression and for production of 2-FL. For pH-stat feeding, the feeding solution was fed automatically into the bioreactor when the pH rose to a value higher than its set-point due to the depletion of glycerol. The pH of medium was controlled at 6.8 by addition of 28% NH_4OH. Agitation speed elevated up to 1,200 rpm for prevention of the deficiency of dissolved oxygen, and air flow rate was maintained at 2 vvm during the fermentation.

2.3. Measurement of concentrations of cell and extracellular metabolites

Dry cell weight (DCW) was determined using optical density and a predetermined conversion factor (0.36). Optical density was measured at 600 nm absorbance using a spectrophotometer (Ultrospec 2000, Amersham Pharmacia Biotech,

USA) after the samples were diluted to keep optical density between 0.1 and 0.5. Extracellular concentrations of 2-FL, lactose, glycerol, galactose and acetic acid were measured by a high performance liquid chromatography (HPLC) (Agilent 1100LC, USA) equipped with the Carbohydrate Analysis column (Rezex ROA-organic acid, Phenomenex, USA) and refractive index (RI) detector. The column heated at 60°C was applied to analyze 20 μl of diluted culture broth. Five millimoles of H_2SO_4 solution was used as a mobile phase at a flow rate of 0.6 mL/min.

2.4. Identification of 2-FL produced by engineered *E. coli* expressing WcfB

2-FL was readily purified and enriched by solid-phase extraction (SPE) using a porous graphitized carbon cartridge prior to mass spectrometry analysis. Enriched 2-FL was analyzed using a Bruker ultrafleXtreme MALDI-TOF/TOF system with 1,000 Hz Smart beam Ⅱ laser. 2, 5-Dihydroxy-benzoic acid (DHB) was used as a matrix (5 mg/100 mL in 50% ACN:H_2O) and 0.01 M NaCl was added as a cation dopant to increase signal sensitivity. The sample was spotted on a stainless steel target plate, followed by the NaCl dopant and matrix. The spot was dried under vacuum prior to mass spectrometric analysis. To confirm the structure of 2-FL, MALDI-TOF/TOF MS via collision-induced dissociation (CID) was performed. Tandem MS spectra were gained at 1k eV collision energy with argon gas.

3. Results

3.1. Exploration of putative α-1,2-fucosyltransferase for producing 2-FL

In spite of previous attempts to increase the expression level and activity of

FucT2 from *H. pylori*, yet the fucose transfer was suggested to be the rate limiting for microbial production of 2-FL. To overcome the barrier, eleven of putative α-1,2-fucosyltransferases were cloned from various origins and expressed in *E. coli* ΔL M15 BCGW which harbors a set of genes for the *de novo* synthesis for GDP-L-fucose (*manB, manC, gmd* and *wcaG*). The eleven candidates were *wcfW* and *wcfB* from *B. fragilis* NCTC9343, *wbsJ* from *E. coli* O128:B12, SPO3391 from *Ruegeria pomeroyi* DSS-3, PGA1_c33070 from *Phaeobacter gallaeciensis* DSM 17395, Phep_1971 from *Pedobacter heparinus* DSM 2366, Pedsa_2797 from *P. saltans* DSM 12145, llmg_2349 from *Lactococcus lactis* subsp. *cremoris* MG1363, Dfer_0178 from *Dyadobacter fermentans* DSM 18053, HMPREF0351_11954 from *Enterococcus faecium* DO, Fut2 from *Homo sapiens*. As a result of flask cultures in LB media with 10 g/L lactose and HPLC analysis, a peak matching with the retention time of 2-FL was detected only in the culture of the strain expressing *wcfB* from *B. fragilis* among the candidate α-1,2-fucosyltransferase genes tested. Although the unknown peaks were also observed in the cultures of the strains expressing PGA1_c33070 from *P. gallaenciensis* and Phep_1971 from *P. heparinus*, the retention time of the peaks were different from 2-FL (data not shown). In the case of the cultures of the strains expressing the other genes, a peak was not observed. It might be due to problems with gene expression or the level of 2-FL concentration might be below the detection limit.

3.2. Identification of 2-FL produced by engineered *E. coli* expressing WcfB

To verify production of 2-FL in the culture of engineered *E. coli* ΔL M15 BCGW-W expressing *wcfB* from *B. fragilis*, a subsequent analysis was performed by MALDI-TOF MS. As shown in Fig. 2a, the ions at 511.16 corresponding to 2-FL

($[Hex_2+Fuc_2+Na]^+$) were observed as a major oligosaccharide in the culture medium. Hexose series consisting of the degree of polymerization 6 to 9 were also present as minor oligomers of 2-FL production. Tandem mass spectrometry (MS/MS) using collision-induced dissociation (CID) was further performed to confirm the composition and to elucidate the structure of 2-FL. The representative MS/MS spectrum with the ion at *m/z* 511.16 was shown in Fig. 2b. CID yields the loss of a fucose at *m/z* 511.16, followed by the loss of a second fucose at *m/z* 365.016, and the loss of a hexose at *m/z* 203.032. Indeed, it was clearly verified the presence of 2-FL produced by engineered *E.coli* expressing WcfB. Interestingly, di-fucosyllactose was not detected in the culture of engineered *E. coli* expressing WcfB contrary to the case of engineered *E. coli* expressing FucT2 from *H. pylori* (Fig. 2c and 2d).

3.3. Effects of replacement of FucT2 with WcfB on 2-FL production

After the confirmation of 2-FL production in *E. coli* expressing WcfB, flask cultures of the engineered *E. coli* ΔL M15 expressing FucT2 and the engineered *E. coli* ΔL M15 expressing WcfB were performed in defined medium containing 20 g/L glycerol and 10 g/L lactose for comparing the 2-FL production performance. As a result, 0.85 g/L of 2-FL was produced in the culture of the engineered *E. coli* expressing WcfB (Fig. 3b). This value was 10 fold higher than that of the engineered *E. coli* expressing FucT2 (Fig. 3a, b and Table 2). The 2-FL production performance of the engineered *E. coli* ΔL M15 BCGW-W was also assessed in fed-batch fermentation in a 2.5 L bioreactor under the same condition as done for previous research (Chin et al., 2015). By consumption of glycerol, cell mass reached up to 56.2 g/L of final cell concentration. As a result, 10.3 g/L of 2-FL with a yield of 0.53 g 2-FL/g lactose and productivity of

0.255 g/L/h were obtained at the end of fed-batch fermentation (Fig 4a and Table 3). These values were 4.0, 8.4 and 5.9 fold higher than those of the ΔL M15 BCGW-F.

3.4. Host strain improvement: complete deletion of *lacZ*

Ideally, carbon flux toward cell growth and GDP-L-fucose synthesis is provided from the glycerol supplementation, thus a theoretical maximum yield of 2-FL from lactose is predicted to be 1.0 mole 2-FL/mole lactose. However, the experimental yield of 2-FL from the culture of ΔL M15 BCGW-W was only about 37.1% of the theoretical yield. This result indicates that more than 60% of the lactose consumed was used for other purposes such as cell growth and by-products formation. Instead of *LacZ*-attenuation, the *lacZ*-deleted mutant ΔL YA was employed to improve the 2-FL yield from lactose. As a result, *E. coli* ΔL YA BCGW-W (*lacZ*-deleted mutant) produced 1.33 g/L of 2-FL in batch fermentation with a yield of 0.83 mole 2-FL/mole lactose, which are 1.6- and 2.8 times higher than those of the ΔL M15 BCGW-W (*lacZ*-attenuated mutant) strain (Fig. 3b, c and Table 2).

Fed-batch fermentation of *E. coli* ΔL YA BCGW-W was also performed to increase the final concentration of 2-FL (Fig. 4b). The growth pattern and the final dry cell weight of *E. coli* ΔL YA BCGW-W were similar to that of the ΔL M15 BCGW-W. After addition of lactose and IPTG, 2-FL concentration increased sharply up to 15.4 g/L in 46 h culture. The 2-FL yield from lactose and productivity were 0.60 mole/mole lactose and 0.530 g/L/h, which were 1.6- and 2.1 folds higher than those from the ΔL M15 BCGW-F strain, respectively (Table 3).

3.5. Scale up: pilot scale production of 2-FL

Fed-batch fermentation of *E. coli* ΔL YA BCGW-W was performed in a 75 L bioreactor in order to demonstrate the feasibility of mass production of 2-FL. The key objective was to reproduce 2-FL titer, productivity and yield as obtained in a laboratory scale bioreactor (2.5 L). The fermentation conditions such as media components, inoculum size, feeding strategy and pH were the same as those of the laboratory scale bioreactor. However, a temperature of the main culture before IPTG induction was changed to 37°C because the cells did not grow well at 25°C (data not shown). The agitation speed and aeration were controlled manually because the dissolved oxygen (DO) is dependent on cell density. As shown in Fig. 5, the DO value dropped rapidly from 100% to 9.2% during the batch period (0 - 8 h) as dry cell weight increased from 0.4 g/L to 5.2 g/L. After depletion of initial 20 g/L of glycerol, the culture temperature was changed to 25°C and the DO value was restored to 73%. Finally, 12.3 g/L of 2-FL concentration was achieved with a yield of 0.591 g 2-FL/g lactose at the end of fed-batch fermentation. Compared to 2.5 L scale fermentation, 80% of the final concentration of 2-FL was obtained in the fermentation done in a 75 L bioreactor.

4. Discussion

Through a screening process, putative α-1,2-fucosyltransferase of *B. fragilis*, WcfB was selected to replace FucT2 from *H. pylori*. The *wcfB* gene which is one of the extracellular polysaccharide biosynthesis genes in *B. fragilis* has been predicted as a gene coding for glycosyltransferase, especially fucosyltransferase due to the identity and similarity to α-1,2-fucosyltransferase in *Yersinia enterocolitica* O8 and *Homo*

sapiens (Comstock et al., 1999). By the expression of WcfB in replacement of FucT2, 2-FL concentration and yield were improved by 4.0- and 8.4-folds in fed-batch fermentation (Table 3). It is assumed that the *in vivo* fucosylation activity of WcfB is much higher than that of FucT2 in the engineered *E. coli*. It is noteworthy that WcfB does not catalyze the biosynthesis of di-fucosyllactose contrary to FucT2 (Fig. 2c and d). It might be due to the difference in enzyme specificity between FucT2 and WcfB. This feature is a great advantage in commercial production of 2-FL since purification is one of the most costly steps in microbial production of value-added materials (Sauer et al., 2008).

It has been reported that 170 mg/L of maximum GDP-L-fucose concentration was obtained in the fed-batch fermentation of the engineered *E. coli* expressing the GDP-L-fucose biosynthetic genes (*manB*, *manC*, *gmd*, *wcaG*) without expressing α-1,2-fucosyltransferase (Lee et al., 2009). This value is corresponded to only 141 mg/L 2-FL since 1 mole of GDP-L-fucose is needed to produce 1 mole of 2-FL. However, 15.4 g/L 2-FL (corresponded to 18.5 g/L GDP-L-fucose) was produced by expressing WcfB in this study. It was reported that GDP-L-fucose is an intracellular metabolite and causes feedback inhibition to the Gmd enzyme because GDP-L-fucose acts as a competitive inhibitor of GDP-D-mannose (Somoza et al., 2000; Sturla et al., 1997). Thus, achievement of 15.4 g/L 2-FL could be due to alleviation of the feedback inhibition by shifting GDP-L-fucose through the biosynthesis of 2-FL.

Interestingly, the protein band corresponding to the predicted size of WcfB was not observed in SDS-PAGE analysis and no fucosylation activity was detected in an *in vitro* fucosylation assay (data not shown). This result is consistent with the previous

research which reported that the purified WcfB enzyme did not accept lactose as a substrate at all in an *in vitro* fucosylation activity assay (Albermann et al., 2001). Therefore, the WcfB enzyme might be considered to be active only in the cell.

The fermentation performance in a 75 bioreactor is inferior to that in a 2.5 L bioreactor, which is an inherent problem associated with scale-up of fermentation processes. Especially, the 2-FL yield based on lactose consumed decreased to 0.591 g 2-FL/g lactose in a 75 L bioreactor from 0.858 g 2-FL/g lactose in a 2.5 L bioreactor (Table 3). More research will be necessary to reproduce the fermentation performance in a large-scale fermentation system. 2-FL concentration, yield and productivity were enhanced further by 50%, 62% and 100% through the complete deletion of *lacZ*. This results is consistent with our previous research done using the engineered *E. coli* strain able to produce 2-FL from fucose, lactose and glycerol via the *salvage* GDP-L-fucose biosynthetic pathway (Chin et al., 2016). It might be due to a shift of the lactose flux from cell growth to 2-FL production. Although β-galactosidase activity was completely eliminated, the percent yield (experimental yield/theoretical yield) was 60%. This result suggests that approximately 40% lactose consumed might be used for by-products formation. Characterization and engineering of WcfB as well as eliminating by-products formation would lead to an improvement of 2-FL titer and yield further.

Acknowledgements

This work was supported by The Advanced Biomass R&D Center (ABC) of Korea Grant (2011-0031359) and Danish Research Council (0652-20140008).

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Figure legends

Fig. 1. Strategy for production of 2-FL in engineered *E. coli*. The names of enzymes are abbreviated as follows; LacY, lactose permease; LacZ, β-galactosidase; ManA, mannose 6-phosphate isomerase; ManB, phosphomannomutase; ManC, mannose 1-phosphate guanylyl-transferase; Gmd, GDP-D-mannose-4,6-dehydratase; WcaG, GDP-4-keto-6-deoxymannose 3,5-epimerase 4-reductase; WcfB, α-1,2-fucosyltransferase. PPi and GTP denote diphosphate and guanosine 5'-triphosphate.

Fig. 2. Representative MS spectrum of 2-FL production from ΔL YA BCGW-W identified by MALDI-TOF MS (a). CID tandem MS spectrum of mono-FL ($[Hex_2+Fuc_1+Na]^+$) at *m/z* 511.164 (b). Purified fucosyllactoses from engineered *E. coli* expressing WcfB (c) and expressing FucT2 (d). Symbols for the types of monosaccharaides (glucose, ●; galactose, ■; fucose, ▲)

Fig. 3. Effects of complete deletion of *lacZ* on 2-FL production in batch fermentations of (a) ΔL M15 BCGW-F, (b) ΔL M15 BCGW-W and (c) ΔL YA BCGW-W. When optical density (OD_{600}) reached 0.8, IPTG and lactose was added to a final concentration 0.1 mM and 10 g/L, respectively (arrow). Symbols are denoted as follows: dry cell weight, ●; lactose, ■; 2-FL, ▲; glycerol, ◆.

Fig. 4. Comparison of 2-FL production of engineered *E. coli* ΔL M15 BCGW-W (a) and ΔL YA BCGW-W (b) in fed-batch fermentation. After depletion of 20 g/L glycerol,

glycerol pH-stat was started. IPTG and lactose were also added at the same time (arrow). Symbols are denoted as follows: dry cell weight, ●; lactose, ■; 2-FL, ▲; glycerol, ◆; acetate, ▼.

Fig. 5. Fed-batch fermentation of engineered *E. coli* ΔL YA BCGW-W done in pilot scale bioreactor. After depletion of 20 g/L glycerol, glycerol pH-stat was started. IPTG and lactose were also added at the same time (thick arrow). In addition, temperature was shifted from 37°C to 25°C. Symbols are denoted as follows: dry cell weight, ●; lactose, ■; 2-FL, ▲; glycerol, ◆; acetate, ▼; dissolved oxygen (DO), ─

Fig. 1

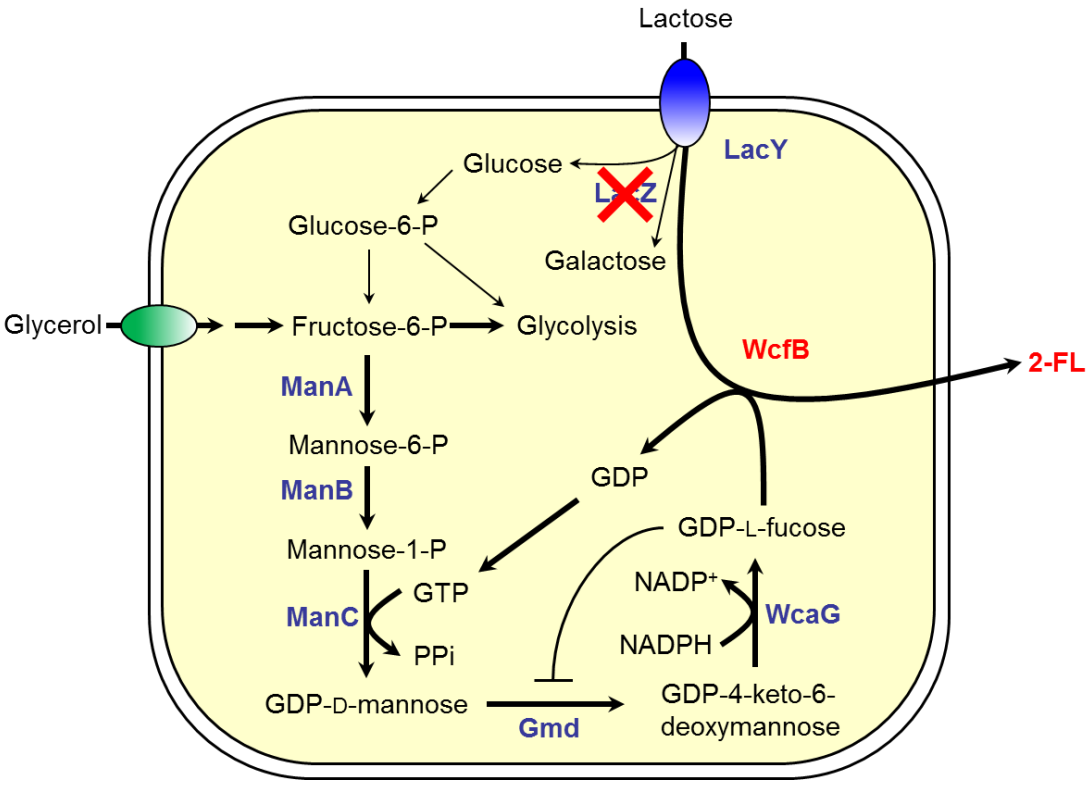


Fig. 2

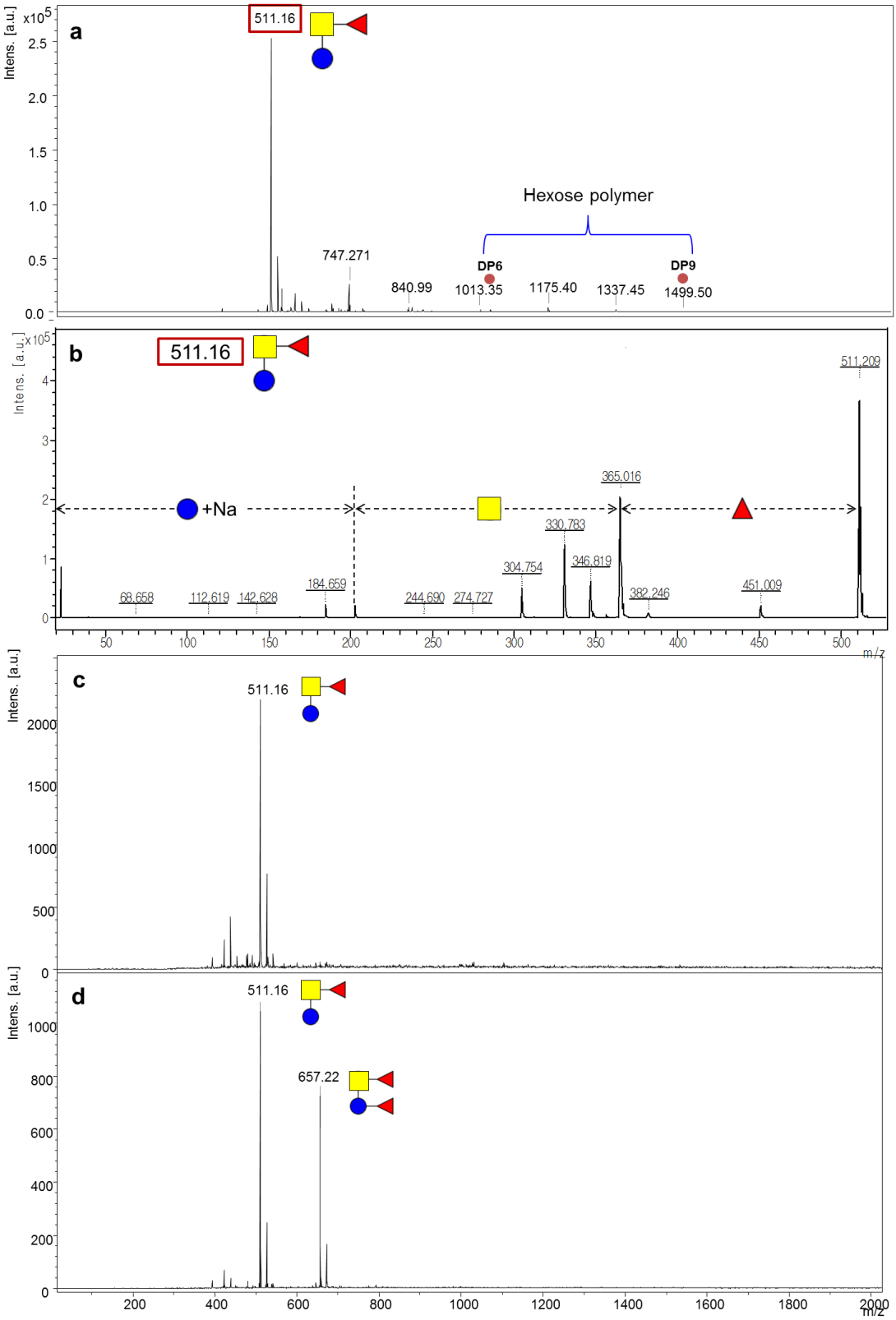
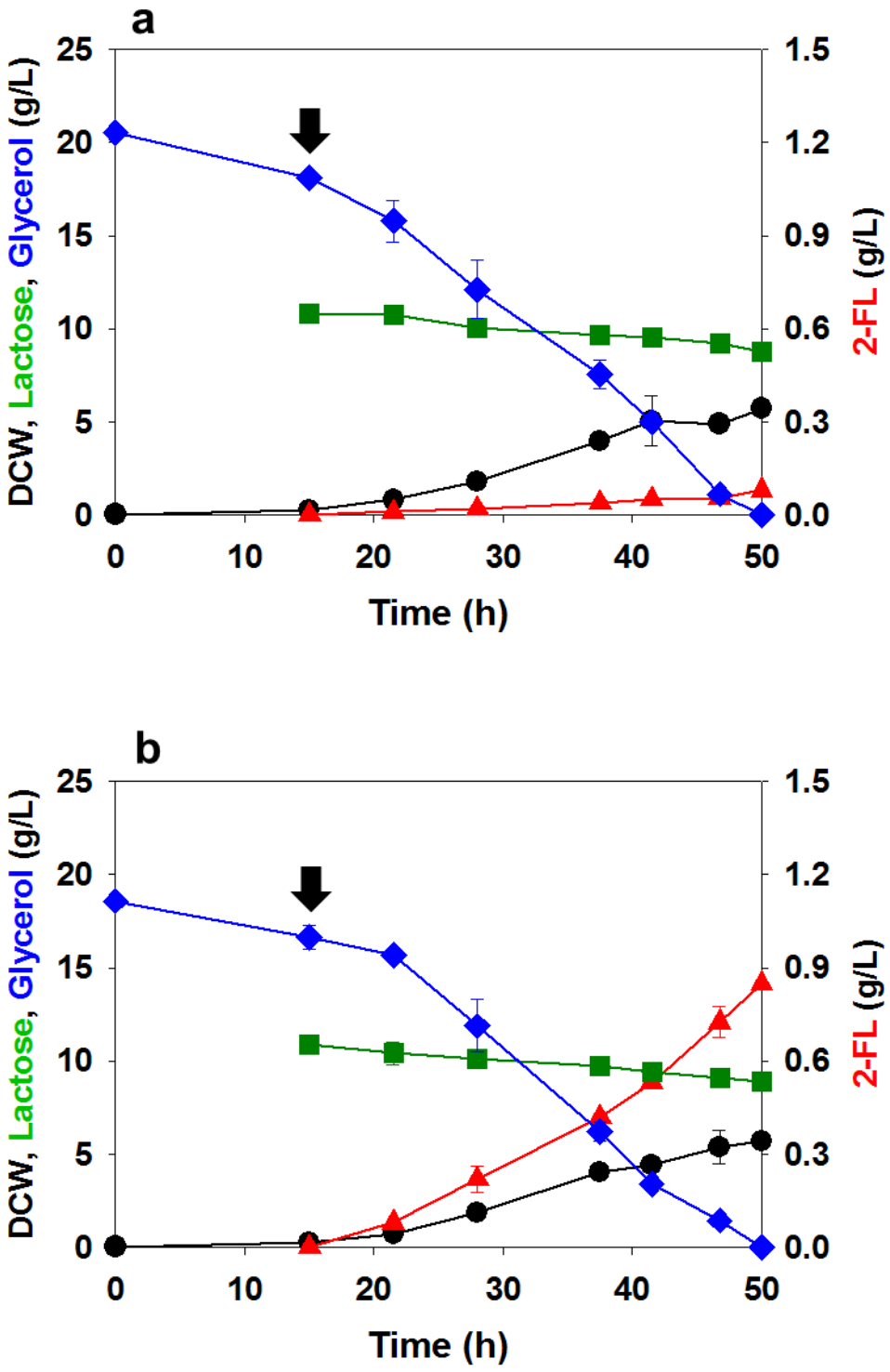


Fig. 3



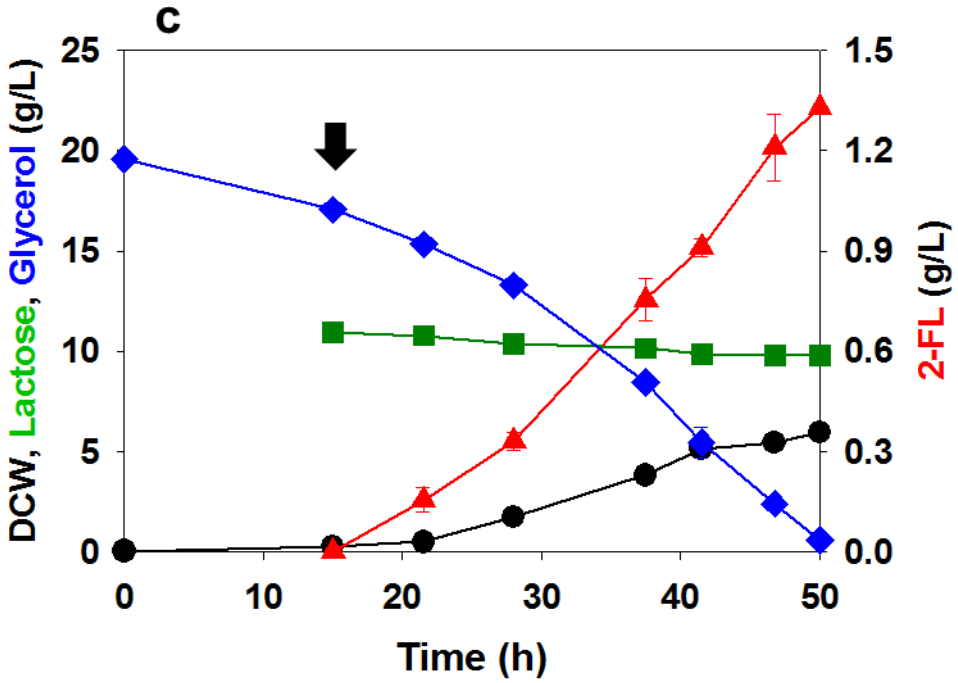


Fig. 4

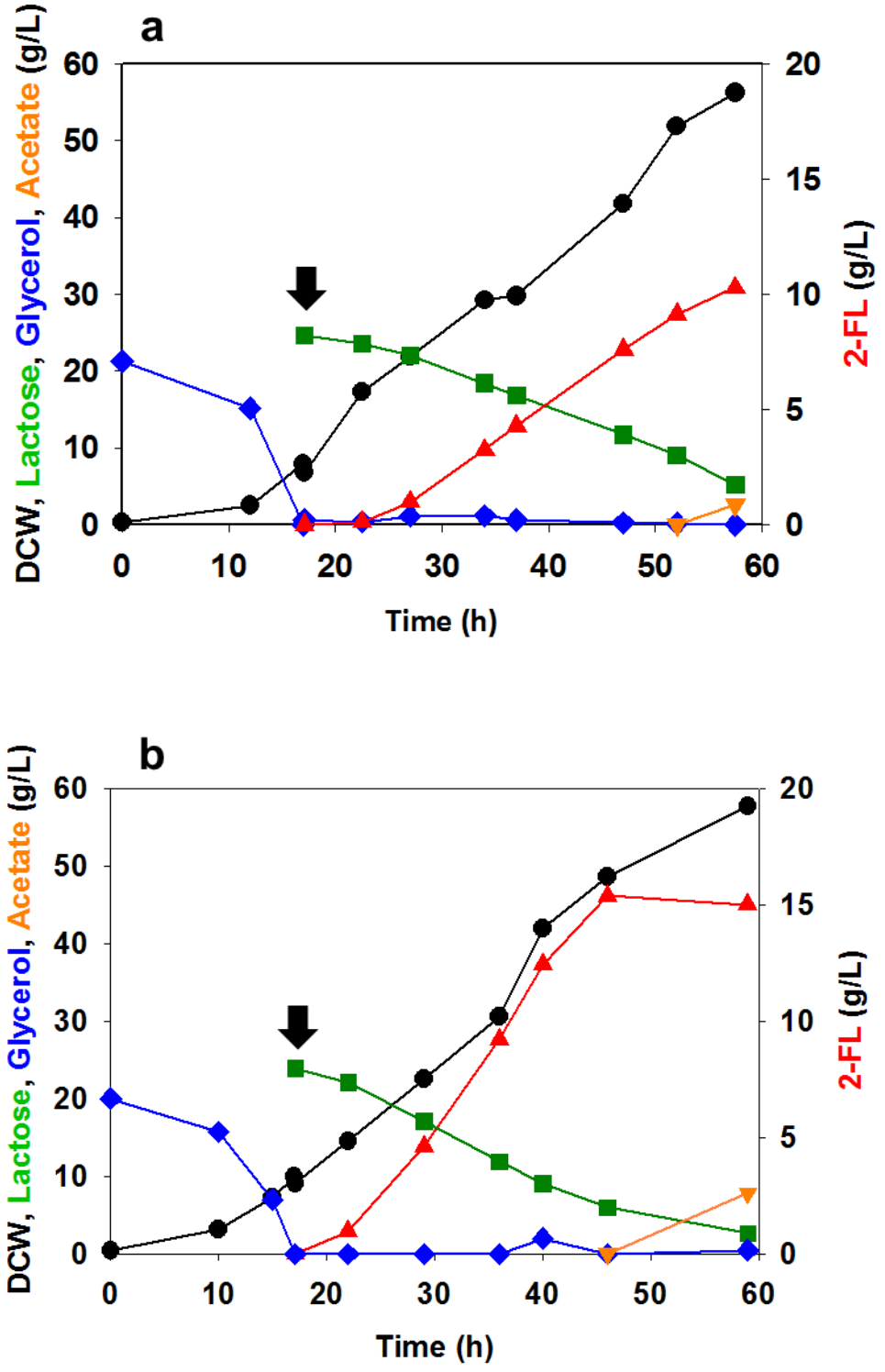


Fig. 5

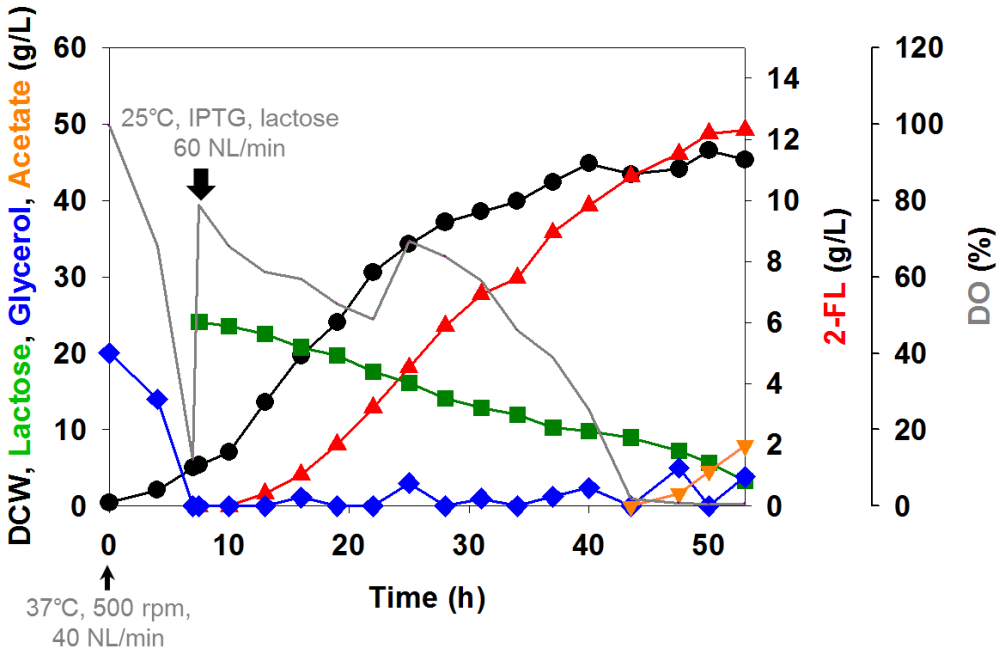


Table 1 List of strains and plasmids used in this study

Strains/Plasmids	Relevant description	Reference
Strains		
ΔL M15	ΔL *Tn7::lacZΔM15YA* (*lacZ* partially disrupted)	(Chin et al., 2015)
ΔL YA	ΔL *Tn7::lacYA* (*lacZ* completely deleted)	(Chin et al., 2016)
ΔL M15 BCGW-F	ΔL M15 harboring pBCGW (*manB*, *manC*, *gmd*, *wcaG*) and pF (*fucT2*)	(Chin et al., 2015)
ΔL M15 BCGW-W	ΔL M15 harboring pBCGW (*manB*, *manC*, *gmd*, *wcaG*) and pBFW (*wcfB*)	This study
ΔL YA BCGW-W	ΔL YA harboring pBCGW (*manB*, *manC*, *gmd*, *wcaG*) and pBFW (*wcfB*)	This study
Plasmids		
pETDuet-1	Two T7 promoters, pBR322 replicon, Amp^R	Novagen
pCOLADuet-1	Two T7 promoters, ColA replicon, Kan^R	Novagen
pBCGW	pETDuet-1 + *manC-manB* (*Nco*I/*Sac*I) + *gmd-wcaG* (*Nde*I/*Kpn*I)	(Lee et al., 2009)
pBFW	pCOLADuet-1 + *wcfW* (CAH07600.1) from *Bacteroides fragilis*	This study
pBFB	pCOLADuet-1 + *wcfB* (CAH06753.1) from *Bacteroides fragilis*	This study
pECJ	pCOLADuet-1 + *wbsJ* (AAO37698.1) from *Escherichia coli* O128 : B12	This study
pRPS	pCOLADuet-1 + SPO3391 (AAV96618.1) from *Ruegeria pomeroyi* DSS-3	This study
pPGP	pCOLADuet-1 + PGA1_c33070 (AFO92946.1) from *Phaeobacter gallaeciensis* DSM 17395	This study

pPHP	pCOLADuet-1 + Phep_1971 (ACU04179.1) from *Pedobacter heparinus* DSM 2366	This study
pPSP	pCOLADuet-1 + Pedsa_2797 (ADY53338.1) from *Pedobacter saltans* DSM 12145	This study
pLLL	pCOLADuet-1 + llmg_2349 (CAL98913.1) from *Lactococcus lactis* subsp. cremoris MG1363	This study
pDFD	pCOLADuet-1 + Dfer_0178 (ACT91449.1) from *Dyadobacter fermentans* DSM 18053	This study
pEFH	pCOLADuet-1 + HMPREF0351_11954 (AFK59578.1) from *Enterococcus faecium* DO	This study
pHSF	pCOLADuet-1 + Fut2;Sec2 (AAC24453.1) from *Homo sapiens*	This study

Table 2 Comparison of results of batch fermentations of the engineered *E. coli* strains

Strains	Maximum dry cell weight (g/L)	Maximum 2-FL concentration[a] (g/L)	Yield (mole 2-FL/mole lactose)
ΔL M15 BCGW-F	5.73±0.09	0.08±0.012	0.040±0.003
ΔL M15 BCGW-W	6.00±0.13	0.85±0.015	0.296±0.003
ΔL YA BCGW-W	5.90±0.28	1.33±0.022	0.825±0.012

The values in the table are averages determined from three independent experiments and standard deviations are shown.

[a] Extracellular concentrations of lactose and 2-FL were determined and used for the calculation of lactose consumption and 2-FL production.

Table 3 Comparison of results of fed-batch fermentations of the engineered *E. coli* strains

Strains	Maximum dry cell weight (g/L)	Maximum 2-FL concentration[a] (g/L)	Yield (g 2-FL/g lactose)	Productivity[b] (g/L/h)
ΔL M15 BCGW-F[c]	73.1	2.6	0.063	0.043
ΔL M15 BCGW-W	56.2	10.3	0.530	0.255
ΔL YA BCGW-W	57.6	15.4	0.858	0.530
ΔL YA BCGW-W (75 L)	46.5	12.3	0.591	0.270

[a] Extracellular concentrations of lactose and 2-FL were determined and used for the calculation of lactose consumption and 2-FL production.
[b] 2-FL productivity was estimated during the 2-FL production period after IPTG induction and lactose dumping.
[c] The result of fed-batch fermentation of engineered *E. coli* ΔL M15 expressing *manB*, *manC*, *gmd*, *wcaG* and *fucT2* (from *H. pylori*) was cited in Chin *et al*. (2015).