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ORIGINAL PAPER

Metabolic engineering of *Corynebacterium glutamicum* to produce GDP-L-fucose from glucose and mannose

Young-Wook Chin · Jin-Byung Park · Yong-Cheol Park · Kyoung Heon Kim · Jin-Ho Seo

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Abstract Wild-type *Corynebacterium glutamicum* was metabolically engineered to convert glucose and mannose into guanosine 5′-diphosphate (GDP)-L-fucose, a precursor of fucosyl-oligosaccharides, which are involved in various biological and pathological functions. This was done by introducing the *gmd* and *wcaG* genes of *Escherichia coli* encoding GDP-D-mannose-4,6-dehydratase and GDP-4-keto-6-deoxy-D-mannose-3,5-epimerase-4-reductase, respectively, which are known as key enzymes in the production of GDP-L-fucose from GDP-D-mannose. Coexpression of the genes allowed the recombinant *C. glutamicum* cells to produce GDP-L-fucose in a minimal medium containing glucose and mannose as carbon sources. The specific product formation rate was much higher during growth on mannose than on glucose. In addition, the specific product formation rate was further increased by coexpressing the endogenous phosphomanno-mutase gene (*manB*) and GTP-mannose-1-phosphate guanylyl-transferase gene (*manC*), which are involved in the conversion of mannose-6-phosphate into GDP-D-mannose. However, the overexpression of *manA* encoding mannose-6-phosphate isomerase, catalyzing interconversion of mannose-6-phosphate and fructose-6-phosphate showed a negative effect on formation of the target product. Overall, coexpression of *gmd*, *wcaG*, *manB* and *manC* in *C. glutamicum* enabled production of GDP-L-fucose at the specific rate of 0.11 mg g cell^{-1} h^{-1}. The specific GDP-L-fucose content reached 5.5 mg g cell^{-1}, which is a 2.4-fold higher than that of the recombinant *E. coli* overexpressing *gmd*, *wcaG*, *manB* and *manC* under comparable conditions. Well-established metabolic engineering tools may permit optimization of the carbon and cofactor metabolisms of *C. glutamicum* to further improve their production capacity.

Keywords GDP-L-fucose · GDP-D-mannose · *Corynebacterium glutamicum* · Guanosine nucleotide

Y.-W. Chin · J.-H. Seo (✉)
Department of Agricultural Biotechnology,
Center for Food and Bioconvergence, Seoul National University,
Seoul 151-921, Republic of Korea
e-mail: jhseo94@snu.ac.kr

J.-B. Park
Department of Food Science and Engineering,
Ewha Womans University, Seoul 120-750,
Republic of Korea

Y.-C. Park
Department of Advanced Fermentation Fusion Science
and Technology, Kookmin University, Seoul 136-702,
Republic of Korea

K. H. Kim
School of Life Science and Biotechnology,
Korea University, Seoul 136-713, Republic of Korea

Introduction

Human milk is very unique in respect of its complex oligosaccharides. It contains not only lactose and D-galactose, but also various oligosaccharides [1]. Among those, fucosyloligosaccharides play key roles in various biological processes including pathogenesis, inflammation, tumor metastasis and host immune response modulation [2–4]. The growing recognition of the roles of fucose-containing oligosaccharides in fundamental biological processes has generated a large need for overproduction of fucosyloligosaccharides.

Enzymatic fucosylation of oligosaccharides requires guanosine 5′-diphosphate (GDP)-L-fucose as a donor of L-fucose [5, 6]. However, efficient synthesis of GDP-L-fucose

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is difficult at the moment. Although biotechnological production of GDP-L-fucose using recombinant *Escherichia coli* was reported, its specific content remained at 2.3 mg g cell^{-1} [4, 7].

Microbial synthesis of GDP-L-fucose was suggested to be influenced by a number of factors. One of them was to supply GDP-D-mannose, a precursor of GDP-L-fucose (Fig. 1). Combinatorial overexpression of the GDP-D-mannose biosynthetic genes such as *manA*, *manB* and *manC* resulted in an increase of GDP-L-fucose production in *E. coli* [7]. Another factor is availability of guanosine nucleotides, which served as cofactor in the conversion of mannose-1-phosphate to GDP-D-mannose (Fig. 1). There were two strategies reported for improving supply of guanosine nucleotides in the cells. Overexpression of guanosine-inosine kinase (Gsk) in recombinant *E. coli* led to an improvement of 58 % in GDP-L-fucose production [8]. The other approach was to use permeabilized cells of *Corynebacterium ammoniagenes*, which allows regeneration of the guanosine nucleotides during whole-cell biotransformation with recombinant *E. coli* [9].

Corynebacterium glutamicum is known as a powerful biocatalyst for the production of nucleotides such as guanosine 5′-monophosphate (GMP) and inosine 5′-monophosphate (IMP) [10, 11]. This suggests that intracellular concentrations and thus availability of guanosine nucleotides in *Corynebacterium* spp. would be high compared to conventional bacterial cells (e.g., *E. coli*). In addition, *C. glutamicum* was reported to have a high regeneration capacity of NADPH [12–14], which is required as cofactor during synthesis of GDP-L-fucose from glucose (Fig. 1). Therefore, the Gram-positive bacterium *C. glutamicum* was engineered as a GDP-L-fucose producer in this study.

As the GDP-L-fucose biosynthetic pathway does not exist in wild-type *C. glutamicum*, key genes for GDP-L-fucose production, encoding GDP-D-mannose-4,6-dehydratase (Gmd) and GDP-4-keto-6-deoxy-D-mannose-3,5-epimerase-4-reductase (WcaG) were introduced into *C. glutamicum* ATCC 13032. Afterwards, endogenous *manA*, *manB* and *manC* genes, which are involved in GDP-D-mannose biosynthesis, were additionally overexpressed to increase availability of GDP-D-mannose in the cells.

Materials and methods

Strains and plasmids

All strains, plasmids, and oligonucleotides used are listed in Tables 1 and 2. *E. coli* TOP10 (Invitrogen, Carlsbad, CA, USA) was used for construction of plasmid DNA. *C. glutmamicum* ATCC 13032 (Korean Agricultural Culture Collection, Korea) was used as a host strain for production of GDP-L-fucose. Expression of the *gmd* and *wcaG* genes was controlled under a *tac* promoter in a *C. glutamicum*/*E. coli* shuttle vector, pEKEx2. The *gmd–wcaG* gene cluster from the genomic DNA of *E. coli* K12 (ATCC10798) was amplified using oligonucleotides (F_*Kpn*I-RBS-gmd and R_*Sac*I-wcaG) by the polymerase chain reaction (PCR). The PCR product was digested with *Kpn*I and *Sac*I, and then ligated into pEKEx2 digested with the same restriction enzymes to construct pEGW. Expression of the *manA*, *manB* and *manC* genes was controlled under the same promoter, *tac* in plasmid pVWEx2. To obtain the pVmB, pVmBC and pVmABC vectors, *manA*, *manB* and *manC* genes were amplified from the genomic DNA of *C. glutamicum* ATCC 13032 using the F_*Spe*I-manA, R_*Bam*HI-manA, F_*Pst*I-manB, R_*Bam*HI-*Spe*I-*Xba*I-manB, F_*Xba*I-manC and R_*Spe*I-manC primers, respectively. Amplified *manB* and pVWEx2 were digested with *Pst*I and *Bam*HI, and ligated together for construction of pVmB. Amplified *manC* was digested with *Xba*I and *Spe*I, and ligated into pVmB cut with the same restriction enzymes to obtain pVmBC. To obtain pVmABC, amplified

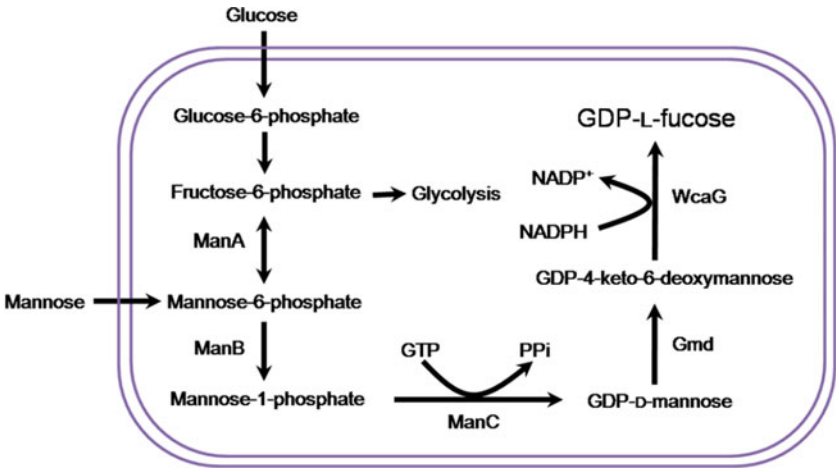

Fig. 1 *De novo* biosynthetic pathway of GDP-L-fucose. ManA, mannose-6-phosphate isomerase; ManB, phosphomannomutase; ManC, GTP-mannose-1-phosphate guanylyltransferase; Gmd, GDP-D-mannose-4,6-dehydratase; WcaG, GDP-4-keto-6-deoxymannose-3,5-epimerase-4-reductase

Table 1 List of strains and plasmids used in this study

Strains/plasmids	Relevant description	Reference
E. coli TOP10	F-*mcr*AΔ (*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139Δ (*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G	Invitrogen (Carlsbad, CA, USA)
C. glutamicum	Wild-type strain, ATCC 13032	
pEKEx2	Km^R; *C. glutamicum*/*E. coli* shuttle vector for regulated gene expression (P_{tac}, $lacI^q$, pBL1, $oriV_{C.g.}$, $oriV_{E.c.}$)	[27]
pVWEx2	Tc^R; *C. glutamicum*/*E. coli* shuttle vector for regulated gene expression (P_{tac}, $lacI^q$, pHM1519, $oriV_{C.g.}$, $oriV_{E.c.}$)	[28]
pEGW	pEKEx2 + *gmd–wcaG*	This study
pVmB	pVWEx2 + *manB*	This study
pVmBC	pVWEx2 + *manB* + *manC*	This study
pVmABC	pVWEx2 + *manA* + *manB* + *manC*	This study

Table 2 Primers used in this study

Name	Sequence
F_*Kpn*I-gmd	GG**GGTACC***AAGGAGATATACA*ATGTCAAAAGTCGCTCTCATCACC
R_*Sac*I-wcaG	C**GAGCTC**TTACCCCCGAAAGCGGTCTTG
F_*Pst*I-manB	AA**CTGCAG***AAGGAGATATACA*ATGCGTACCCGTGAATCTGTCAC
R_*Bam*HI-*Spe*I-*Xba*I-manB	CG**GGATCC**GG**ACTAGT**GC**TCTAGA**TTATGCGCGGATAATCCCTA
F_*Xba*I-manC	GC**TCTAGA***AAGGAGATATACA*ATGACTTTAACTGACAAC
R_*Spe*I-manC	GG**ACTAGT**CTACTGATCAGACGAAAA
F_*Spe*I-manA	GG**ACTAGT***AAGGAG*ATATACAATGGAGCTATTGGAAGGCTC
R_*Bam*HI-manA	CG**GGATCC**CTAAACCCTAGCGAGGAATAC

The bold nucleotides indicate the recognition sites of specific restriction enzymes

The italic sequences present the ribosome binding site (RBS) and spacer

manA was digested with *Spe*I and *Bam*HI, and ligated into the pVmBC cut with the same restriction enzymes. All constructs were proved by the restriction enzyme digestions and DNA sequencing. Transformation of *C. glutamicum* was performed by electroporation [12]. In order to construct recombinant *C. glutamicum* harboring pEgw together with another vector, the cells were first transformed with pEGW [15].

Culture conditions

Escherichia coli strains for construction of plasmids were grown in Luria–Bertani (LB) medium (1 % tryptone, 0.5 % yeast extract, 1 % NaCl) containing appropriate antibiotics (kanamycin 50 μg ml^{-1} or tetracycline 15 μg ml^{-1}) at 37 °C and shaken at 250 rpm. For transformation of *C. glutamicum*, the growth medium containing 25 μg ml^{-1} kanamycin or 5 μg ml^{-1} tetracycline was used. Flask cultures of recombinant *C. glutamicum* strains were performed in a 500-ml baffled flask (Nalgene) containing 100 ml of CGXII minimal medium [16] with 20 g l^{-1} D-glucose, 20 g l^{-1} D-mannose and appropriate antibiotics at 30 °C with 250 rpm agitation. Batch fermentation was carried out in a 2.5-l bioreactor (Kobiotech, Incheon, Korea) containing 1.0 l of CGXII medium containing with 20 g l^{-1} D-glucose, 20 g l^{-1} D-mannose and appropriate antibiotics at 30 °C with 1,000 rpm agitation and 2 vvm aeration. When dry cell weight (DCW) reached approximately 1 g l^{-1}, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration 1.0 mM for induction of gene expression regulated by the *tac* promoter. The pH of medium was controlled at 7.0 using a standard pH electrode (Mettler Toledo, Columbus, OH, USA) by addition of 28 % ammonia and 2 N HCl.

Analytical methods

Dry cell weight was determined using optical density and a predetermined conversion factor. Optical density was measured at 600 nm absorbance using a spectrophotometer (Ulftrospec 2000, Amersham Pharmacia Biotech, Piscataway, NJ, USA) after the samples were diluted to keep optical density between 0.1 and 0.5.

Concentrations of glucose, mannose, lactic acid 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 millimole of H_2SO_4 solution was used as a mobile phase at a flow rate of 0.6 ml min^{-1}.

To determine the concentrations of intracellular GDP-L-fucose, the collected cells were resuspended in extraction buffer containing 50 mM of Tris–HCl (pH 7.5), 150 mM NaCl, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol and 5 mM EDTA [7]. The cell suspensions were placed into 2-ml screw-cap tubes together with 250 mg of 150- to 212-μm glass beads (Sigma-Aldrich, USA) and subjected to mechanical disruption (6 times for 5 min) by a VORTEX-GENIE2 (Scientific industries inc., Bohemia, NY, USA) with intermittent cooling on ice for 2 min [17]. After the disruption, the samples were boiled for 2 min and then glass beads and cellular debris were removed by centrifugation. The supernatants were used for the HPLC analysis. The HPLC (Agilent 1200LC, USA) system equipped with a Capcell Pak C18 MG column (Shiseido Co., Tokyo, Japan) and multiple wavelength detector (MWD) at a flow rate of 0.6 ml min^{-1} and 254 nm. A mobile phase was composed of 20 mM triethylammonium acetate (pH 6.0) and acetonitrile. The intracellular guanosine nucleotides were also quantified by HPLC [18]. The cells were harvested after 12 h seed and the supernatant was obtained by the same method for extraction of GDP-L-fucose. The supernatant was injected into HPLC equipped with the Partisil 10 SAX anion exchange column (250 × 4.6 mm, Whatman, Clifton, NJ, USA). A column was eluted at a flow rate of 1.5 ml min^{-1} by the following gradient program: 100 % eluent A (7 mM KH_2PO_4 at pH 4.0) and 0 % eluent B (250 mM KH_2PO_4 and 500 mM KCl at pH 4.5) for 15 min; 0–100 % eluent B over 45 min; 100 % eluent B over 40 min. Guanosine nucleotides were detected by absorbance at 254 nm.

Total, soluble and insoluble protein fractions were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE, 12 % polyacrylamide gel). The cells resuspended in 500 μl ice-cold TES buffer (25 mM Tris/HCl, 25 mM EDTA, 10.3 % sucrose, pH 8.0) were disrupted using an ultrasonic processor (Cole-Parmer, Vernon Hills, IL, USA) at 75–100 W. After collections of the supernatants (soluble fraction) and cell debris (insoluble fraction) by centrifugation (12,000 rpm, 4 °C, 10 min), each of 12-μl protein fractions were loaded onto the gel and visualized by staining the gels with Coomassie brilliant blue solution.

Results

Intracellular guanosine nucleotides' concentrations of *C. glutamicum*

Guanosine-5′-triphosphate (GTP) is a cofactor required for the conversion of mannose-1-phosphate to GDP-D-mannose, a precursor of GDP-L-fucose (Fig. 1). The amounts of intracellular guanosine nucleotides in *C. glutamicum* and *E. coli* were measured for the cells grown in the BHI medium. A significant amount of GTP was detected in *C. glutamicum*, while little observed in *E. coli* BL21 Star (DE3) (Table 3). The concentrations of GMP and GDP in *C. glutamicum*, which were the precursors of GTP, were also markedly higher than those in the *E. coli* cells. These results indicated that *C. glutamicum* would be a promising host for the production of GDP-L-fucose from glucose and/or mannose.

Table 3 Quantitative analysis of intracellular concentrations of guanosine nucleotides

Strain	GMP	GDP	GTP
E. coli BL21 Star (DE3)	0.10	0.32	N.D.
C. glutamicum ATCC13032	0.84	0.44	0.14

The unit of intracellular guanosine nucleotides is mg g $cell^{-1}$

Expression of *gmd* and *wcaG* in *C. glutamicum*

To construct recombinant *C. glutamicum* that is able to produce GDP-L-fucose from glucose and/or mannose, the key genes *gmd* and *wcaG* from *E. coli* were introduced into *C. glutamicum*. Expression of the two genes was examined via flask cultivation of recombinant *C. glutamicum* harboring pEgw and pEKEx2. Cell extracts from the samples harvested 14 h after 1.0 mM IPTG induction were analyzed by SDS-PAGE. As shown in Fig. 2, the apparent molecular weight of Gmd and WcaG was estimated to be 42 and 36 kDa, corresponding to the theoretical values based on their amino acid sequences. On the other hand, the control strain containing pEKEx2 showed no relevant bands. Furthermore, most of Gmd and WcaG were expressed as soluble fractions in the cells.

Production of GDP-L-fucose by recombinant *C. glutamicum* expressing Gmd and WcaG

The medium containing both glucose and mannose was reported to improve the productivity of GDP-L-fucose compared with the case with either glucose or mannose in recombinant *E. coli* experiments [7]. Therefore, recombinant *C. glutamicum* harboring pEgw and pEKEx2 were cultivated in CGXII minimal medium containing both glucose and mannose as carbon sources (Fig. 3). The fermentation profiles of the two strains were very similar. Glucose was consumed followed by mannose, exhibiting typical diauxic growth. Lactate and acetate accumulated in the medium due to oxygen limitation in flask cultivations. However, introduction of the *gmd* and *wcaG* genes allowed the recombinant *C. glutamicum* pEGW to produce GDP-L-fucose to a final concentration of 31.1 mg l^{-1}. The specific

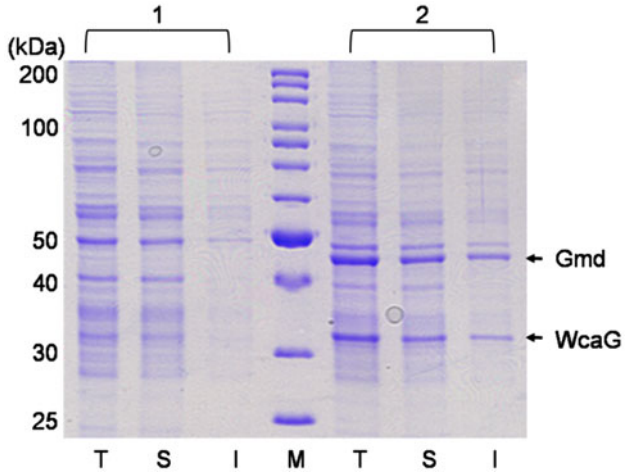

Fig. 2 SDS-PAGE analysis of the cell-free extracts of recombinant *C. glutamicum* harboring pEKEx2 (*1*) and pEgw (*2*). 12 µl of total (T), soluble (S), insoluble (I) proteins were loaded onto 12 % gel. *Lane M* shows a protein size marker

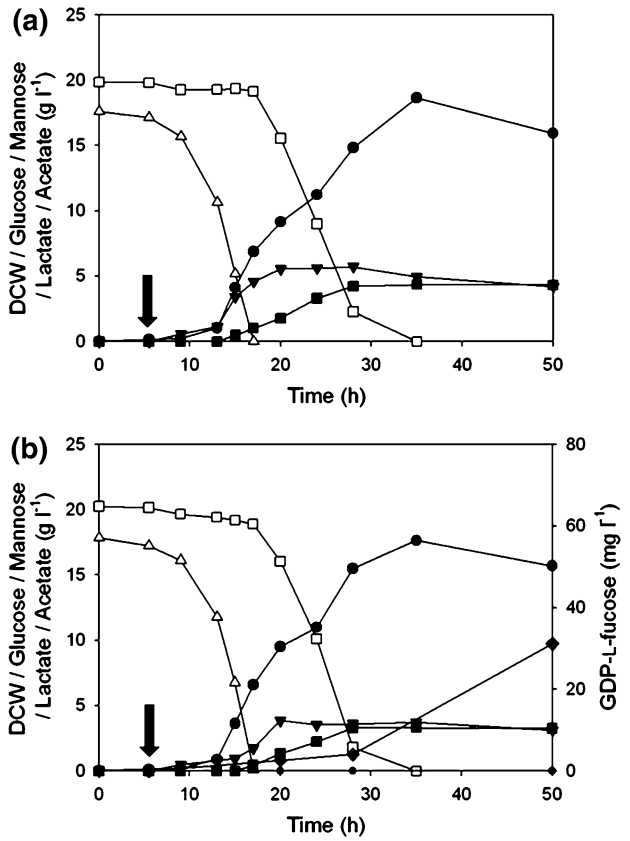

Fig. 3 Production of GDP-L-fucose in recombinant *C. glutamicum* harboring pEKEx2 (**a**) or pEgw (**b**). The *arrow* indicated the time of induction with 1.0-mM IPTG. Symbols indicated dry cell weight (*filled circle*), glucose (*open triangle*), mannose (*open square*), lactate (*filled down triangle*), acetate (*filled square*), and GDP-L-fucose (*filled diamond*)

Table 4 Summary of flask cultures of recombinant *C. glutamicum*

Strains	Dry cell weight (g l^{-1})	GDP-L-fucose concentration (mg l^{-1})	Specific GDP-L-fucose content (mg g $cell^{-1}$)	Specific GDP-L-fucose production rate (mg g $cell^{-1}$ h^{-1})
WT	15.9	ND	ND	ND
GW	15.7	31.1	1.98	0.04
GWmB	12.7	23.0	1.82	0.04
GWmBC	11.8	63.7	5.42	0.12
GWmABC	13.0	60.4	4.66	0.10

GDP-L-fucose content reached 1.98 mg g $cell^{-1}$ (Table 4). The specific GDP-L-fucose production rate was much higher during growth on mannose than on glucose.

Improvement of GDP-L-fucose production by overexpression of GDP-D-mannose biosynthetic enzymes

GDP-D-mannose is a crucial intermediate for production of GDP-L-fucose and the conversion of fructose-6-phosphate to GDP-D-mannose was catalyzed by ManA, ManB and ManC [7]. At first, ManB (E.C. 5.4.2.8, NCgl0714), which catalyzes the conversion of mannose-6-phosphate to mannose-1-phosphate (Fig. 1), was coexpressed in recombinant *C. glutamicum* harboring pEGW. As shown in Figs. 3b and 4a, coexpression of ManB with Gmd and WcaG resulted in a reduction of cell growth rate and final cell mass concentration. Uptake rates of glucose and mannose were also decreased by 10.4 and 32.2 %, respectively. However, the specific GDP-L-fucose content remained similar to the recombinant *C. glutamicum* harboring pEGW only (Table 4).

Additional expression of ManC (E.C. 2.7.7.13, NCgl0710) in recombinant *C. glutamicum* expressing ManB, Gmd, and WcaG did not exert a significant impact on cell growth rate and final cell density (Fig. 4b; Table 4). However, the specific production rate of GDP-L-fucose was increased to 0.12 mg g $cell^{-1}$ h^{-1} and the specific GDP-L-fucose content reached 5.4 mg g $cell^{-1}$, which is a 2.7- and 3.0-fold higher than the recombinant *C. glutamicum* expressing Gmd and WcaG only. The final GDP-L-fucose concentration thereby increased up to 63.7 mg l^{-1}. This indicated that coexpression of *manB* and *manC* along with *gmd* and *wcaG* played a key role in the microbial production of GDP-L-fucose.

We also investigated the effect of coexpression of ManA (E.C. 5.3.1.8, NCgl0716), which catalyzes conversion of fructose-6-phosphate into mannose-6-phosphate (Fig. 1). The overexpression of *manA* led to an increase of final cell

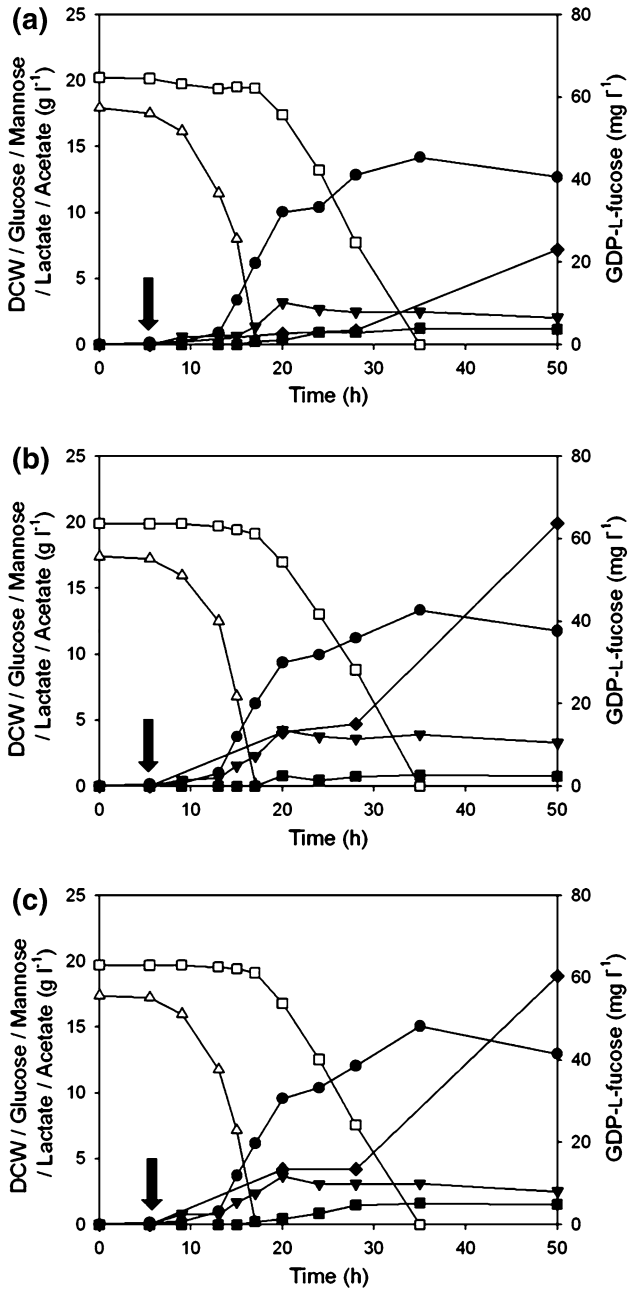

Fig. 4 Effect of coexpression of the GDP-D-mannose biosynthetic enzymes [e.g., ManB (**a**), ManBC (**b**), ManABC (**c**)] with Gmd and WcaG on production of GDP-L-fucose in recombinant *C. glutamicum*. The *arrow* indicated the time of induction with 1.0 mM IPTG. *Symbols* indicated dry cell weight (*filled circle*), glucose (*open triangle*), mannose (*open square*), lactate (*filled down triangle*), acetate (*filled square*), and GDP-L-fucose (*filled diamond*)

density, whereas the specific production rate and specific content of GDP-L-fucose were decreased to 0.10 mg g cell^{-1} h^{-1} and 4.66 mg g cell^{-1}, respectively. This could be due to the fact that overexpression of *manA* resulted in an increase of carbon flux into glycolysis relative to the biosynthetic pathway of GDP-L-fucose, as observed in an earlier study [19].

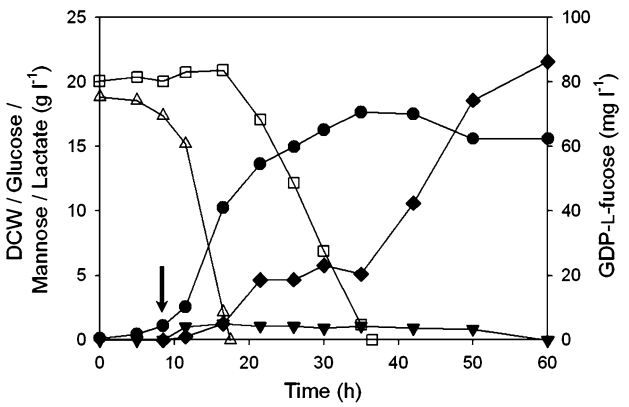

Fig. 5 Production of GDP-L-fucose during batch fermentation of the recombinant *C. glutamicum* harboring pEgw and pVmBC. Aeration and agitation speed were set at 2 vvm and 1,000 rpm, respectively. The *arrow* indicated the time of induction with 1.0-mM IPTG. *Symbols* indicated dry cell weight (*filled circle*), glucose (*open triangle*), mannose (*open square*), lactate (*filled down triangle*) and GDP-L-fucose (*filled diamond*)

Production of GDP-L-fucose in a bioreactor

The fermentation products such as lactate and acetate accumulated in the medium because of oxygen limitation due to insufficient aeration in flask cultivations (Fig. 4). To provide better growth conditions, a bioreactor was employed for batch fermentation of recombinant *C. glutamicum* overexpressing Gmd, WcaG, ManB and ManC (Fig. 5). As expected, sufficient aeration led to a decrease of production of lactate and acetate and hence allowed 32 % higher final cell density than in the flask culture as shown in Fig. 4b. Although the specific production rate and specific content of GDP-L-fucose remained similar to the flask cultivation (Tables 4, 5), the final GDP-L-fucose concentration increased up to 86.2 mg l^{-1}. This was due to higher cell density, which was driven by more efficient oxygen supply in a bioreactor.

Discussion

Corynebacterium glutamicum was metabolically engineered to produce GDP-L-fucose from glucose and mannose. Introduction of the *gmd* and *wcaG* genes of *E. coli* into *C. glutamicum* ATCC 13032 allowed synthesis of GDP-L-fucose from glucose and mannose. Although coexpression of *manB* with *gmd* and *wcaG* did not result in an improvement of GDP-L-fucose production, coexpression of *manB* and *manC* along with *gmd* and *wcaG* led to a significant increase in the specific production rate and specific content of GDP-L-fucose (Table 4). ManB was reported to be subjected to inhibition by its reaction product, mannose-1-phosphate [20] (Fig. 1). Therefore,

Table 5 Comparison of summarized results of fermentations of recombinant *C. glutamicum* and *E. coli*

Strains	Fermentation type	Dry cell weight ($g\ l^{-1}$)	GDP-L-fucose concentration ($mg\ l^{-1}$)	Specific GDP-L-fucose content ($mg\ g\ cell^{-1}$)	Specific GDP-L-fucose production rate ($mg\ g\ cell^{-1}\ h^{-1}$)	
C. glutamicum GWmBC	Batch	15.6	86.2	5.53	0.11	This study
E. coli GWmBC	Fed-batch	74.2	170.3	2.30	0.09	[7]

coexpression of *manC*, which product is involved in conversion of mannose-1-phosphate into GDP-D-mannose and thereby attenuation of the product inhibition as well as increase of substrate availability to Gmd (Fig. 1), could be essential in improving the specific GDP-L-fucose production rate and specific GDP-L-fucose content.

Another interesting point was that the production of GDP-L-fucose from glucose and mannose was associated with growth in the recombinant *E. coli* [7]. However, the product formation in recombinant *C. glutamicum* was not associated with growth in particular, during growth on mannose (Fig. 5). The genus *Corynebacterium* has a unique cell wall structure, which contains glycolipids (e.g., phosphatidyl-myo-inositol, mannosides) and lipoglycans (e.g., lipomannan, lipoarabinomannan) [21, 22]. As GDP-D-mannose is a key substrate in the synthesis of lipomannan [23–25], GDP-D-mannose, which is also an intermediate of GDP-L-fucose, could be preferentially used for synthesis of cell wall during cell growth. Thereby, the GDP-D-mannose flux into production of GDP-L-fucose could be increased only after cell growth ceased in recombinant *C. glutamicum*, exhibiting non-growth association of GDP-L-fucose production.

The specific GDP-L-fucose productivity and specific GDP-L-fucose content of recombinant *C. glutamicum* increased up to 0.11 mg g $cell^{-1}$ h^{-1} and 5.5 mg g $cell^{-1}$, which is 22 % and 2.4-fold greater than the recombinant *E. coli* overexpressing *gmd*, *wcaG*, *manB* and *manC* under comparable conditions (Table 5). These values seemed to be possible by a number of factors. One of them might be a high intracellular concentration of guanosine nucleotides as well as a large capacity of NADPH regeneration in *C. glutamicum* cells as shown in Table 3 and in our previous studies [13, 26]. Engineering of the GDP-D-mannose metabolism to increase its flux into GDP-L-fucose production in *C. glutamicum* would lead to an improvement of GDP-L-fucose productivity further.

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