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Effect of deregulation of repressor-specific carbon catabolite repression on carbon source consumption in *Escherichia coli*

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Abstract

Escherichia coli has been used as a host to construct the cell factory for biobased production of chemicals from renewable feedstocks. Because galactose is found in marine biomass as a major component, the strategy for galactose utilization in *E. coli* has been gained more attention. Although galactose and glucose co-fermentation has been reported using the engineered *E. coli* strain, few reports have covered fermentation supplemented with galactose as a sole carbon source in the mutant lacking the repressor-specific carbon catabolite repression (CCR). Here, we report the effects of the deregulation of the repressor-specific CCR (*galR*$^-$ and *galS*$^-$) in fermentation supplemented with galactose as a sole carbon source, using the engineered *E. coli* strains. In the fermentation using the *galR*$^-$ and *galS*$^-$ double mutant (GR2 strain), an increase of rates in sugar consumption and cell growth was observed compared to the parent strain. In the glucose fermentation, wild-type W3110 and its mutant GR2 and GR2PZ (*galR*$^-$, *galS*$^-$, *pfkA*$^-$, and *zwf*$^-$) consumed sugar at a higher rate than those values obtained from galactose fermentation. However, the GR2P strain (*galR*$^-$, *galS*$^-$, and *pfkA*$^-$) showed no difference between fermentations using glucose and galactose as a sole carbon source. This study provides essential information for galactose fermentation using the CCR-deregulated *E. coli* strains.

Keywords: Escherichia coli, Carbon catabolite repression, Glucose, Galactose, Galactose operon

Introduction

The production of industrially essential compounds from sustainable biomass is attracting attention as a critical technology for solving serious global problems, including climate change [1–4]. *Escherichia coli* strains have been widely used as a host for developing the cell factory to produce chemicals from sustainable biomass [5–10]. One reason is the rich and well-defined information on genetics, physiology, and tools, for metabolic rewiring by engineering [11–13].

Meanwhile, to avoid competition with food in bio-based chemical production, non-edible biomass is also receiving more attention. Thus, efforts have been made to utilize various biomass and control the consumption of carbon sources [14]. *E. coli* uses numerous sugars as a carbon source. However, when *E. coli* cells were exposed to the environment presenting glucose and other sugars, a primarily prefer glucose is used first for cell growth in the organism by carbon catabolite repression (CCR) mechanism [15–17]. The CCR mechanism is a facility for rapid growth in competition with other microorganisms in nature. In the CCR mechanism, carbon source catabolic genes are regulated by the combination of the phosphotransferase system (PTS), catabolite activator protein (CAP), and repressor-specific regulatory mechanisms [18].

Galactose is assumed as a valuable carbon source found in marine macroalgae for bio-based chemical production [19]. For this reason, our recent study has reported

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a strategy to control two carbon source consumption in the glucose and galactose co-fermentation by altering the CCR mechanism in *E. coli* [20]. In the previous report, mutant *E. coli* strains GR2 (*galR*$^-$, *galS*$^-$), GR2P (*galR*$^-$, *galS*$^-$, *pfkA*$^-$), and GR2PZ (*galR*$^-$, *galS*$^-$, *pfkA*$^-$, *zwf*$^-$) were intensively characterized focusing on the co-fermentation of glucose and galactose [20]. However, these mutant *E. coli* strains have not yet been characterized in fermentations supplemented with sole carbon source. This can be critical for flexible managing in the replacement of feedstocks depending on the industrial supply and demand situation of carbon sources in fermentation using the *E. coli* strains in which the CCR has been engineered. In this study, we examined the effect of the deregulation of the repressor-specific CCR on fermentation supplemented with either glucose or galactose as a sole carbon source in *E. coli*.

Materials and methods

E. coli strains

In this study, the wild-type *E. coli* W3110 and its mutants GR2, GR2P, and GR2PZ strains were used in order to see the effect of deregulation of repressor-specific CCR. The mutants GR2 (*galR*$^-$ and *galS*$^-$), GR2P (*galR*$^-$, *galS*$^-$, and *pfkA*$^-$), and GR2PZ (*galR*$^-$, *galS*$^-$, *pfkA*$^-$, and *zwf*$^-$) were constructed by knockout of the genes using Lambda Red and Cre recombinases in our previous study [20].

E. coli cell culture and media

For all cultures, *E. coli* cells were incubated in a 500 mL flask containing 220 mL of R/2 medium at 37 °C, 200 rpm the IST-4075 incubator (Jeiotech, Korea). The R/2 medium was prepared by adding 0.85 g/L citric acid, 0.7 g/L $MgSO_4{\cdot}7H_2O$, 2 g/L $(NH_4)_2HPO_4$, and 6.75 g/L KH_2PO_4 in final concentration; five milliliter of trace metal stock (200×) was added per liter. Trace metal stock (200×) was prepared by solving 2.25 g $ZnSO_4{\cdot}7H_2O$, 1 g $CuSO_4{\cdot}5H_2O$, 0.58 g $MnSO_4{\cdot}5H_2O$, 0.1 g $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.02 g $Na_2B_4O_7{\cdot}10H_2O$, 2 g $CaCl_2{\cdot}2H_2O$ and 10 g $FeSO_4{\cdot}7H_2O$ in 1 L of 0.1 M HCl. Six grams of either glucose or galactose were added as the sole carbon source per liter of R/2. Seed cultures were made by inoculating *E. coli* cells from a glycerol stock into 25 mL test tubes containing 10 mL of R/2 medium. After overnight at 37 °C, 200 rpm, the culture was used as inoculum.

Analytical methods

Cell density was observed by determining OD_{600} using the JP/U-1900 spectrophotometer (Hitachi, Japan). In this study, dry cell weight (DCW) was obtained from the conversion of OD_{600}. We estimated that 1.0 OD at 600 nm is equivalent to 0.27 g/L DCW [21]. Glucose and galactose concentrations were measured using the Agilent 1100 series, high-performance liquid chromatography (Agilent Technologies, USA) equipped with the RI-101, refractive index detector (Shodex, Denmark) [22–24]. For LC analysis, flow rate was controlled at 0.5 mL/min using 0.01 N H_2SO_4, mobile phase through the MetaCarb 87H column (Agilent Technologies, USA) at 25℃. Specific sugar consumption rate (g/gDCW/h) was calculated from the sugar consumption from 0 h to an endpoint showing a rapid consumption. For example, the specific glucose consumption rate was determined from the glucose consumption amount until 16 h in the cultures using W3110 strain.

Results and discussion

In a recent report, it was reported that the wild-type *E. coli* W3110 cells first consumed glucose rapidly with a rate of 1.37 g/gDCW/h, followed by galactose consumption with a rate of 0.04 g/gDCW/h in both sugars co-fermentation [20]. This indicates that galactose consumption was strictly inhibited by the CCR mechanism in the W3110 strain (Fig. 1). In this study, to see the effect of the CCR mechanism on consumption rate under sole carbon source fermentation, we determined the specific sugar consumption rate in W3110 cultures supplemented with either 6 g/L glucose or 6 g/L galactose (Fig. 2a, b). In the glucose fermentation, the wild-type W3110 strain grew up to OD_{600} of 1.52 from 5.33 g/L carbon source in 48 h cultures (Fig. 2a), which is very similar to the value obtained in the galactose fermentation (Fig. 2b). However, we found differences in the maximum specific growth rate and the specific sugar consumption rate between glucose- and galactose-fermentations using the W3110 strain (Table 1). The wild-type W3110 strain showed increases in growth and sugar consumption rates in glucose fermentation by 1.41 and 1.37 times, respectively, compared to values in galactose fermentation (Table 1). This result indicates that, although the two carbon sources are not competing conditions, the wild-type W3110 strain favors glucose as a feedstock rather than galactose under the same situation.

Next, we examined the *galR* and *galS* double mutant, GR2 strain, to see the effect of deregulation of repressor-specific CCR on the consumption of glucose and galactose as a sole carbon source (Fig. 2c, d). Galactose operon repressors encoded by the *galR* and *galS* genes are involved in the CCR mechanism by binding to the operator locus (Fig. 1). In the previous report, under the glucose and galactose co-fermentation (each 4 g/L), the *E. coli* GR2 cells showed a similar specific glucose consumption rate of the W3110 strain [20]. However, in the same co-fermentation, the GR2 strain achieved a 3.38 times increase in specific galactose consumption rate [20]. In

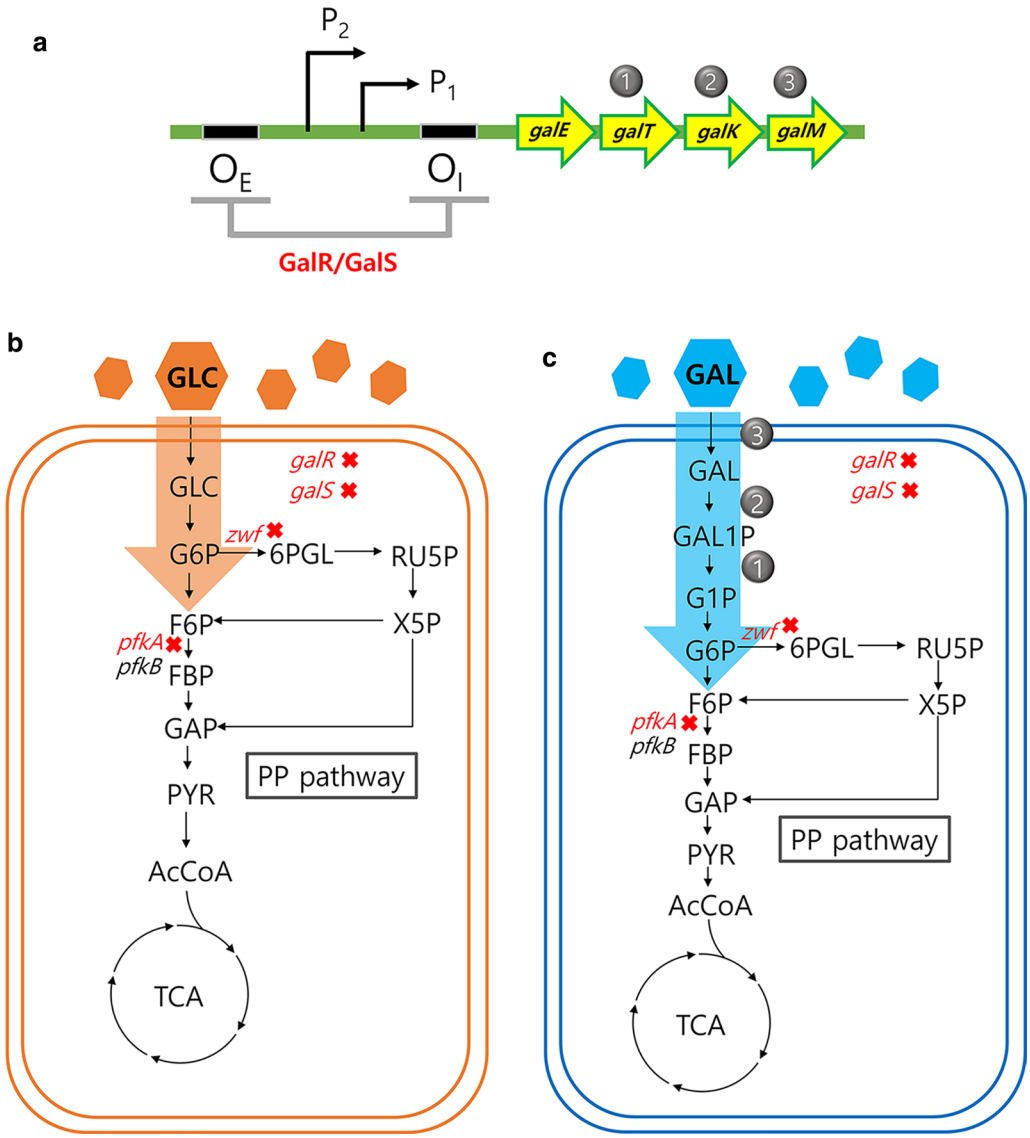

Fig. 1 Schematic overview of pathways for carbohydrate utilization in *E. coli*. **a** Glucose catabolism in *E. coli*. **b** Galactose catabolism in *E. coli*. The "X" indicates deletion of the corresponding gene. Symbols and abbreviations: operators, O_E and O_I; promoters, P_1 and P_2; GLC, glucose; G6P, glucose-6-phosphate; 6PGL, 6-phosphogluconolactone; RU5P, ribulose-5-phosphate; X5P, xylulose-5-phosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-phosphate; GAP, glyceraldehyde phosphate; PYR, pyruvate; AcCoA, acetyl-CoA; PP, pentose phosphate; TCA, tricarboxylic acid; GAL, galactose; GAL1P, galactose-1-phosphate; and G1P, and glucose-1-phosphate. Gene abbreviations for enzyme encoding: *galE*, galactose epimerase; *galT*, galactose-1-phosphate uridylyltransferase; *galK*, galactokinase; *galM*, galactose mutarotase; *galR*, DNA-binding transcriptional repressor; *galS*, DNA-binding transcriptional isorepressor; *pfkA*, 6-phosphofructokinase I; *pfkB*, 6-phosphofructokinase II; and *zwf*, glucose-6-phosphate dehydrogenase

this study, in 6 g/L glucose fermentation, the GR2 strain showed an increase in the specific glucose consumption rate compared with the W3110 strain, but no significant change in the maximum specific growth rate (Table 1). In 6 g/L galactose fermentation, the GR2 strain showed an increase in the specific galactose consumption rate and the maximum specific growth rate compared with the wild-type strain (Table 1). These indicate that the deregulation of repressor-specific CCR positively affected central metabolism in both cultures supplemented with glucose and galactose as the sole carbon source. Especially, the double mutation of *galR* and *galS* genes was more sensitive in galactose fermentation than glucose fermentation. Nevertheless, glucose was still favored as the carbon source by the *E. coli* GR2 strain, in which the GalS and GalS repressor-specific CCR was deregulated.

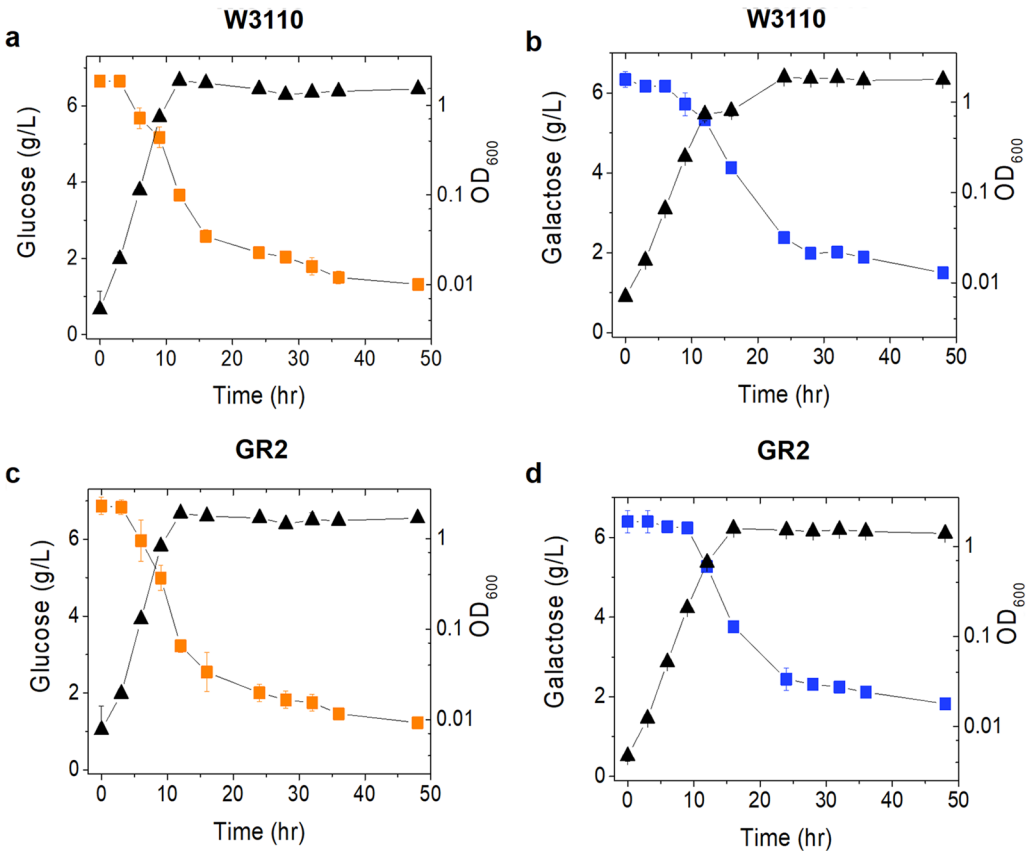

Fig. 2 Fermentation profiles of *E. coli* strains W3110 and GR2 in R/2 medium containing 6 g/L glucose or 6 g/L galactose. Symbols: OD_{600} (black triangles), glucose (orange squares), and galactose (blue squares)

Table 1 Fermentation parameters obtained from the cultures of the wild-type *E. coli* W3110 strain and its mutants GR2, GR2P, and GR2PZ in R/2 medium containing either 6 g/L glucose or 6 g/L galactose

Strain	Maximum specific growth rate (μ_{max}; /h)		Specific sugar consumption rate (g/gDCW/h)[a]	
	Glucose fermentation	Galactose fermentation	Glucose	Galactose
W3110	0.4888 ± 0.05	0.3470 ± 0.00	0.9793 ± 0.08	0.7127 ± 0.04
GR2	0.5098 ± 0.01	0.3643 ± 0.01	1.1777 ± 0.11	0.8644 ± 0.07
GR2P	0.2989 ± 0.00	0.3057 ± 0.01	0.5922 ± 0.03	0.3110 ± 0.02
GR2PZ	0.2196 ± 0.01	0.1649 ± 0.01	0.5344 ± 0.05	ND

ND not determined

[a] Specific sugar consumption rate (g/gDCW/h) was calculated from the sugar consumption from 0 h to an endpoint showing a rapid consumption

To see the effect of additional gene knockout of either *pfkA* or *pfkA/zwf* in the GR2 strain on sugar consumption, we examined the GR2P and GR2PZ strains, in which three (*galR, galS,* and *pfkA*) and four (*galR, galS, pfkA*, and *zwf*) genes were knocked out, respectively (Fig. 3). Interestingly, GR2P strain showed similar maximum specific growth rates of 0.2989 /h and 0.3057 /h in glucose- and galactose-fermentations, respectively. These were resulted from a high similarity between the maximum specific sugar consumption rates on glucose (0.5566 g/gDCW/h) and galactose (0.5234 g/gDCW/h). It seems that the growth of the GR2P strain was delayed by blocking the Embden-Meyerhof-Parnas (EMP) pathway, regardless the type of carbon source. Thus, in the fermentation using *E. coli* GR2P strain, it was also revealed that glucose was not

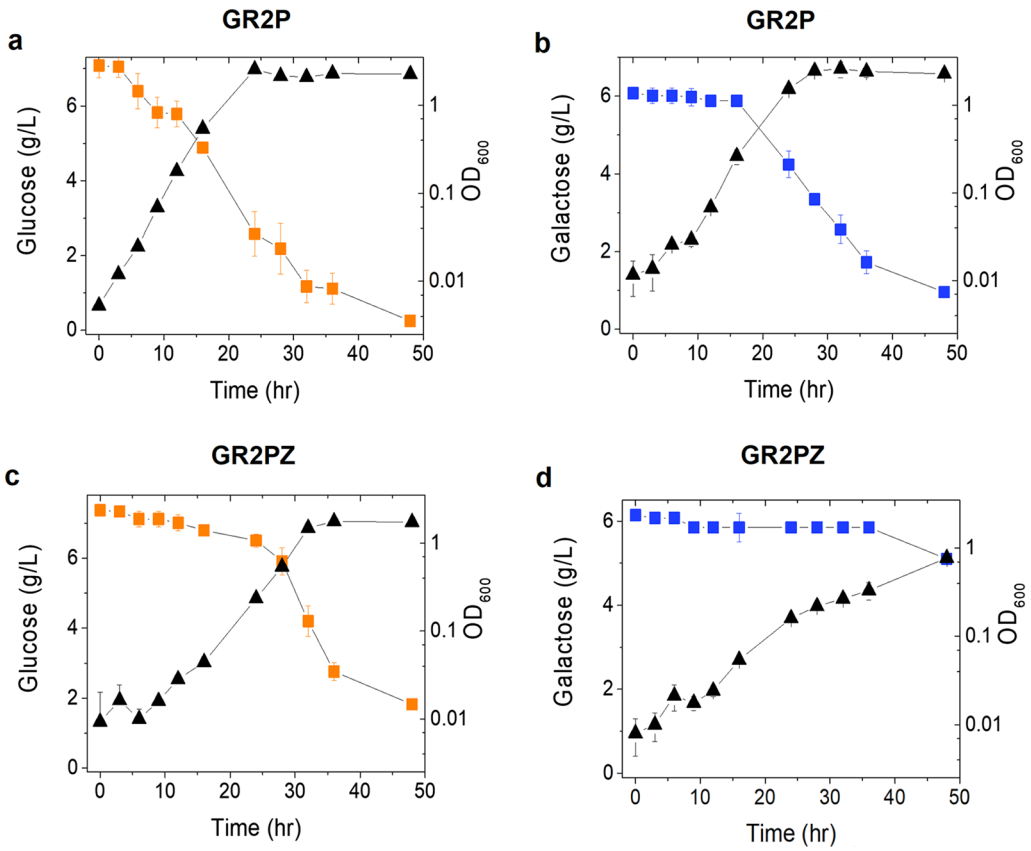

Fig. 3 Fermentation profiles of *E. coli* strains GR2P and GR2PZ in R/2 medium containing 6 g/L glucose or 6 g/L galactose. Symbols: OD_{600} (black triangles), glucose (orange squares), and galactose (blue squares)

further preferred as a sole carbon source compared to galactose. In the GR2P strain, glucose and galactose are primarily catabolized through the pentose phosphate pathway (PPP) at the node of glucose-6-phosphate [25]. In the fermentations using GR2PZ strain, in which the PPP was further blocked by the *zwf* gene knockout together with disruption of *galR, galS*, and *pfkA* genes, the growth was further retarded. However, in such fermentation using GR2PZ strain, the preference for glucose as a carbon source was restored as like to wild-type and GR2 strain. In this study, we characterized the wild-type *E. coli* W3110 strain and its mutants GR2 (galR- and galS-), GR2P (galR-, galS-, and pfkA-), and GR2PZ (galR-, galS-, pfkA-, and zwf-) in sole carbon source fermentation supplemented with either glucose or galactose. In all strains tested in this study except GR2P, glucose was primarily preferred as a sole carbon source rather than galactose. The GR2P strain showed no difference between fermentation using glucose and galactose as a sole carbon source, especially in sugar consumption and cell growth rates. Our study provides critical information for flexible managing in the replacement of feedstocks depending on the industrial supply and demand situation of carbon sources in fermentation using the CCR engineered *E. coli* strains. In this study, we characterized the wild-type *E. coli* W3110 strain and its mutants GR2 ($galR^-$ and $galS^-$), GR2P ($galR^-$, $galS^-$, and $pfkA^-$), and GR2PZ ($galR^-$, $galS^-$, $pfkA^-$, and zwf^-) in sole carbon source fermentation supplemented with either glucose or galactose. In all strains tested in this study except GR2P, glucose was primarily preferred as a sole carbon source rather than galactose. The GR2P strain showed no difference between fermentation using glucose and galactose as a sole carbon source, especially in sugar consumption and cell growth rates. Our study provides critical information for flexible managing in the replacement of feedstocks depending on the industrial supply and demand situation of carbon sources in fermentation using the CCR engineered *E. coli* strains.

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Authors' contributions
YSJ designed the project. HJS performed experiments, and HJS and YSJ analyzed data. HJS and YSJ wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

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