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Production of acetol from glycerol using engineered *Escherichia coli*

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HIGHLIGHTS

- A recombinant *E. coli* strain Lin43 was constructed to convert glycerol into acetol.
- Deletion of the gene of *gloA* increased the acetol yield by 32%.
- Overexpression of the gene of *yqhD* increased the acetol production by 11.4-fold.
- Under the optimal conditions, the engineered strain produced 5.4 g/L acetol in 21 h.

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ABSTRACT

Escherichia coli Lin43 is a strain which has some mutations in glycerol kinase (GlpK) and the repressor for the glycerol 3-phosphate regulon (GlpR). When exposed to glycerol, it quickly accumulates lethal levels of methylglyoxal, which is a precursor of acetol; acetol is important for the manufacture of polyols, acrolein, dyes, and skin tanning agents. This work reports the engineering of *E. coli* Lin 43 for the conversion of glycerol into acetol. First, the glyoxalase system was interrupted by deleting the *gloA* gene, which increased the acetol yield by 32%. In addition, the aldehyde reductase YqhD was overexpressed which led to an increase of acetol production by 11.4-fold. Acetol production was optimized by varying the cell density, glycerol concentration, supplemental carbon source, pH and temperature. Under the optimal conditions (OD_{600} = 20, 20 g/L glycerol, 2 g/L succinate, pH 7.0, and 28 °C), we obtained 5.4 g/L acetol in 21 h.

1. Introduction

Due to the increase in environmental concerns and diminishing supplies of fossil fuels, biodiesel, a good alternative and renewable fuel, has attracted much attention because it could reduce net greenhouse effects (Almeida et al., 2012). As a result, biodiesel production increased rapidly in the past few years. For example, the global biodiesel production in 2010 was about 5 billion gallons, which is 4.7-fold more than that in 2005 (Almeida et al., 2012). However, biodiesel production based on triglycerides will produce about 10% (w/w) glycerol as the main byproduct. The increasing production of biodiesel results in an increased production of glycerol. It was estimated that the global biodiesel market would reach 37 billion gallons by 2016, which means about 4 billion gallons of crude glycerol will be generated. (Yang et al., 2012). The excess of glycerol produced in the biodiesel industry is leading to a decrease in glycerol prices and glycerol is now considered a waste rather than a co-product (Yazdani and Gonzalez, 2007). Therefore, it is imperative to discover potential value-added applications of glycerol and provide an ideal platform for chemical and pharmaceutical industries. A great number of studies have used diverse chemical and biological approaches to convert glycerol to various value-added products (Barbelli et al., 2012; Clomburg and Gonzalez, 2013; Posada et al., 2012; Wang and Yang, 2013).

Acetol, a C3 keto alcohol, is an important intermediate used to produce polyols and acrolein (Mohamad et al., 2011). It is also widely used as a reduced dye in the textile industry and as a skin tanning agent in the cosmetic industry (Soucaille et al., 2008b). Acetol can be synthesized from glycerol via dehydration (Chiu et al., 2008; Yamaguchi et al., 2010) or from propylene glycol via dehydrogenation (Sato et al., 2008). However, the high cost of acetol by chemical processes reduces its industrial application and markets (Soucaille et al., 2008a).

Bioconversion of glycerol is considered to be a more sustainable option, since it offers an environmentally friendly approach without the use of high temperatures/pressures and the addition of toxic organic solvents. However, there are only a limited number of publications for bioconversion of glycerol to acetol. It was reported that three strains of *Clostridium thermosaccharolyticum* could produce acetol from D-xylose and D-glucose, and the highest yield reached was 1.47 g/L (Cameron and Cooney, 1986). Cameron et al. used *Saccharomy cerevisiae* expressing the *mgs*A gene of *Escherichia coli* to produce acetol. However, the best titers reported are

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below 0.1 g/L (Cameron et al., 1999). Moreover, Soucaille et al. claimed that 1.40 and 1.63 g/L of acetol could be obtained respectively by two different genetically engineered *E. coli* strains from glucose (Soucaille et al., 2008a,b).

Glycerol can be transformed to acetol via the methylglyoxal (MG) bypass pathway, in which DHAP is converted to MG and subsequently metabolized to acetol and 1,2-propanediol (Cooper, 1984). MG is very toxic and arrests growth of *E. coli* at millimolar concentrations. Thus, its production is tightly controlled in the cells. In order to improve the metabolic flux through this pathway, it is essential to overexpress MG synthase and the genes converting glycerol to DHAP and at a low phosphate condition (Altaras and Cameron, 1999; Clomburg and Gonzalez, 2011).

E. coli strain Lin43, which lacks fructose-1, 6-bisphosphate inhibition of glycerol utilization, synthesizes excessive MG during unregulated glycerol metabolism (Lin, 1976). As a result, cells are killed because of the accumulation of cytotoxic MG (Freedberg et al., 1971). In microorganisms, MG can be converted to acetol by aldo–keto reductases (Cameron and Cooney, 1986; Ko et al., 2005). *E. coli* Lin43 strain can accumulate MG when the cells are using glycerol as a carbon resource. Therefore, it is feasible to produce acetol from glycerol by strain Lin43 if MG can be rapidly converted to acetol.

The aims of this study were to obtain a genetically engineered Lin43 strain which can efficiently produce acetol from glycerol. By using resting cells, we obtained 5.38 g/L of acetol in 21 h. To our knowledge, this is highest report of production of acetol from glycerol by *E. coli* and the highest yield.

2. Methods

2.1. Strains and plasmids

The strains, plasmids and primers used in this study are listed in the Table 1. For deleting and overexpressing genes, we used the Keio collection and the ASKA library, respectively. Kanamycin (50 μg/mL) was used for pre-culturing the isogenic knock-outs. Chloramphenicol (30 μg/mL) was used for the strains harboring pCA24N and its derivatives. The *gloA* gene in Lin43 was disrupted by P1 phage transduction (Silhavy et al., 1984).

2.2. Culture medium and growth conditions

Cells were cultivated aerobically in medium A containing 34 mM NaH_2PO_4, 64 mM K_2HPO_4, 20 mM $(NH_4)_2SO_4$, 0.3 mM $MgSO_4$, 0.001 mM $FeSO_4$, 0.001 mM $ZnCl_2$, 0.01 mM $CaCl_2$, 15 mM sodium succinate, and 0.04% (g/g) casein hydrolysate (Tanaka et al., 1967). Liquid cultures were routinely incubated in 250-mL-baffled Erlenmeyer flasks at 37 °C and 130 rpm.

2.3. Production of acetol by the resting cells

To obtain the active biomass of the resting cells, the cells were grown in medium A in the presence of the appropriate antibiotics at 37 °C and 180 rpm to an OD_{600} of 0.3 and induced with 1 mM of IPTG. After 3 h, the cells were harvested by centrifugation (5000*g*, 4 °C, and 8 min), washed with phosphate buffer (100 mM, pH 7.0), and resuspended in the phosphate buffer containing glycerol at 20 g/L. Biotransformations were performed at 37 °C and 180 rpm aerobically.

2.4. Analytical methods

Concentrations of acetol, glycerol, and MG were measured by high performance liquid chromatography (HPLC; Agilent, model 1260) using a Rezex ROA-Organic Acid H^+ column (300 mm × 7.8 mm; Phenomenex, Torrence, CA, USA) and a differential refractive index (RI) detector (Altaras and Cameron, 1999). Prior to analysis, each sample was centrifuged at 5000*g* for 8 min and the supernatant was filtered through 0.45 μm membranes. The operation conditions were: mobile phase, 5 mM H_2SO_4; column temperature, 60 °C; flow rate, 0.5 mL/min. The retention time of MG, glycerol and acetol are 16.7 min, 18.2 and 22.7 min, respectively.

3. Results and discussion

3.1. The deletion of gloA gene increased acetol yield

MG, the precursor of acetol, is a toxic metabolite known for its inhibition of cell growth and cell death (Booth et al., 2003; MacLean et al., 2002). There are several pathways for the degradation of MG in *E. coli*. The glyoxalase system, which consists of glyoxalase I (encoded by *gloA*) and II (encoded by *gloB*), is the dominant route to transform MG (MacLean et al., 2002). In order to improve the yield of acetol, the glyoxalase system in Lin43 strain was disrupted by deleting the *gloA* gene. As shown in Fig. 1, the *gloA*-deficient strain reached the yield of acetol as 0.044 g/g glycerol, which was 32% more than that of the parent strain. These results indicate that the disruption of the glyoxalase system improves the carbon flux flow to acetol and improved its yield. Therefore, the Lin43 Δ*gloA* strain was used for the further studies.

Table 1
Strains, plasmids and primers used in this study.

Strains, plasmids primers	Description/genotype/sequence	Source
Strains		
Lin43	Hfr(PO2A) *fhuA22*, *ΔphoA8*, *fadL701*(T2R), *relA1*, *glpR2*(*glp*^{c}), *pitA10*, *spoT1*, *glpK22*(fbR), *rrnB-2*, *mcrB1*, *creC510*	CGSC (Freedberg et al., 1971)
BW25113*ΔgloA*	*lacI*^{q} *rrnB*$_{T14}$ *ΔlacZ*$_{WJ16}$ *hsdR*514 *ΔvaraBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$; deletion mutant for *gloA* gene in BW25113	(Baba et al., 2006)
Lin43 Δ*gloA*	Lin43, Δ*gloA::FRT-km-FRT*; deletion mutant for *gloA* gene in Lin43	This study
Lin43 Δ*gloA*/pCA24N	Lin43 Δ*gloA*, *Cm*; *lacI*^{q}, pCA24N	This study
Lin43 Δ*gloA*/pCA24N-mgsA	Lin43 Δ*gloA*, *Cm*; *lacI*^{q}, pCA24N P$_{T5\text{-}lac}$::*mgsA*$^+$	This study
Lin43 Δ*gloA*/pCA24N-yqhD	Lin43 Δ*gloA*, *Cm*; *lacI*^{q}, pCA24N P$_{T5\text{-}lac}$::*yqhD*$^+$	This study
Plasmids		
pCA24N	*Cm*; *lacI*^{q}, pCA24N	(Kitagawa et al., 2005)
pCA24N-mgsA	*Cm*; *lacI*^{q}, pCA24N P$_{T5\text{-}lac}$::*mgsA*$^+$	(Kitagawa et al., 2005)
pCA24N-yqhD	*Cm*; *lacI*^{q}, pCA24N P$_{T5\text{-}lac}$::*yqhD*$^+$	(Kitagawa et al., 2005)
Primers		
V-gloA	F:5′-GCCGCCGCTATACTAAAACA-3′ R:5′-TGAGCGTTATCGGACATCTG-3′	This study

Cm and *km* are chloramphenicol and kanamycin resistance, respectively.

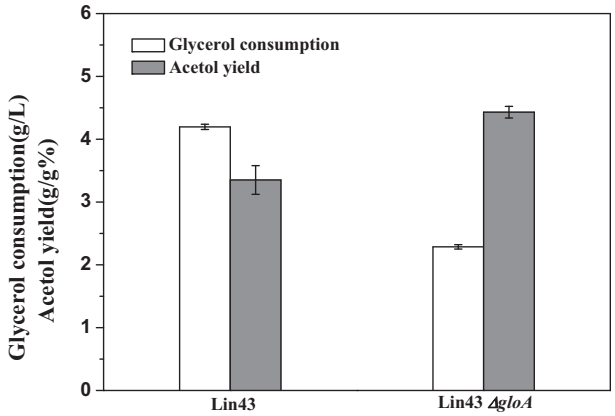

Fig. 1. Production of acetol in strain Lin43 Δ*gloA*. Left: Lin43 strain; right: Lin43 Δ*gloA* strain acetol was produced 24 h after adding glycerol into the growth medium when OD_{600} reached 0.75. Transformation was conducted aerobically at 37 °C.

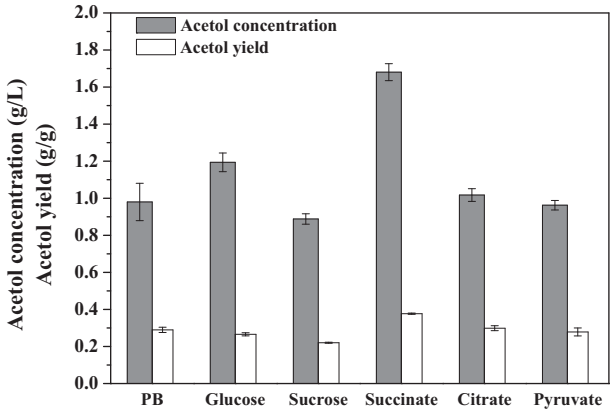

Fig. 3. Effect of supplemental carbon sources on the production of acetol by resting cells of Lin43 Δ*gloA*/pCA24N-yqhD. The samples were analyzed at 24 h after transferring the resting cells into the conversion medium containing 2 g/L succinate (equivalent to approximately 67 mmol C/L) or other carbon sources (equivalent to approximately 67 mmol C/L). The biotransformation was conducted aerobically at 37 °C.

3.2. Overexpression of the genes for the conversion of MG to acetol

MG can be converted to acetol by the action of aldehyde reductase which is encoded by *yqhD* gene (Clomburg and Gonzalez, 2011; Ko et al., 2005), and YqhC is a transcriptional activator of *yqhD* (Lee et al., 2010). In addition, MG can be converted to acetol by aldo–keto reductases (AKRs) from *E. coli* and the mammalian sources (Ko et al., 2005). Some AKRs, such as YafB and AKR from human liver (Cao et al., 1998), have large values of k_{cat}/K_m on MG. Therefore, overexpression of the *yqhD* gene, aldo–keto reductases from *E. coli* (encoded by *yafB*) and human liver (*akr*), and the *yqhC* gene were investigated. As shown in Fig. 2, the concentration of acetol produced by the overexpressed strains increased compared to the control. The yield of acetol by *yqhD* overexpression reached 0.58 g/L, which was 11.4-fold higher than that of the control. Overexpression of *yafB*, *akr*, and *yqhC* also increased the yield by 6.4, 5.2 and 1.8-fold, respectively.

The *E. coli* aldehyde reductase YqhD has drawn the attention of researchers when it was used to produce 1, 3-PD using recombinant *E. coli* (Jarboe, 2011). YqhD has reductase activity for a broad range of short-chain aldehydes (Jarboe, 2011) and has successfully been used for the production of 1,2-propanediol at high yield and titer (Clomburg and Gonzalez, 2011). Our results show that YqhD is more suitable than several kinds of aldo–keto reductase for acetol production. Thus, the strain Lin43 Δ*gloA* pCA24N-yqhD was selected for further study.

3.3. Production of acetol by the resting cells

In order to produce MG from glycerol, strain Lin43 was grown in medium A for full expression of the *glp* operon and then the cells were exposed to glycerol (Freedberg et al., 1971). Moreover, it has reported that high density of cells can increase the resistance to MG (Fraval and McBrien, 1980). Furthermore, there is only one step from MG to acetol. Thus, it is feasible to transform glycerol to acetol using resting cells of Lin43 Δ*gloA* pCA24N-yqhD. First, the possibility of production of the acetol by resting cells was investigated. It was found that the resting cells in phosphate buffer with 20 g/L glycerol could produce 0.51 g/L acetol at OD_{600} = 0.75 (equal to 0.31 g cell dry weight per liter), which is about the same level as the exponentially growing cells (0.58 g/L, Fig. 2). The results indicate that the resting cells do not lose activity for converting glycerol to acetol in phosphate buffer.

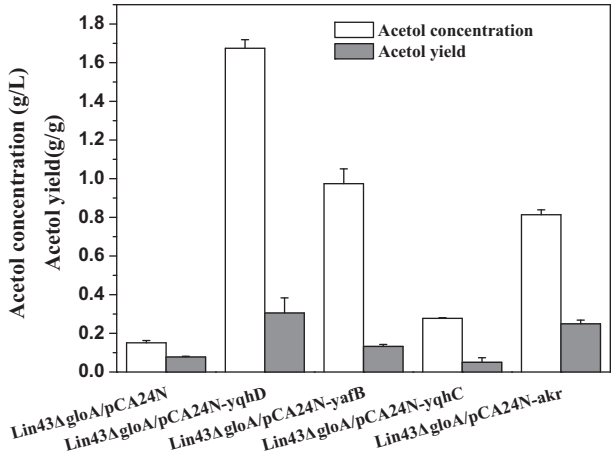

Fig. 2. Effect of overexpression of the gene of *akr*, *yafB*, *yqhC*, and *yqhD* on acetol production in strain Lin43. Glycerol was added to the medium to 20 g/L when OD_{600} reached 0.75, then the samples were analyzed after 24 h aerobic incubation at 37 °C.

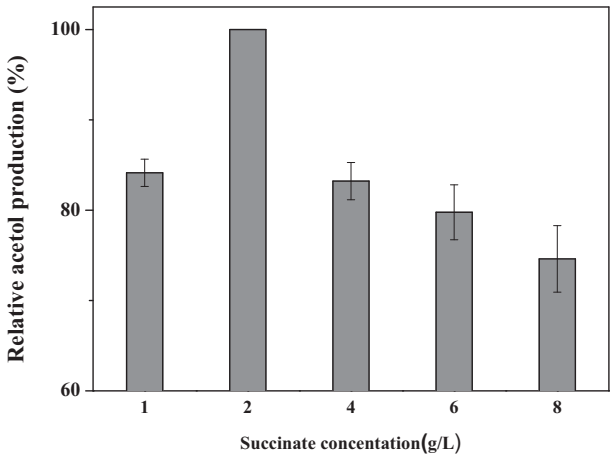

Fig. 4. Effect of succinate concentration on the production of acetol by resting cells of Lin43 Δ*gloA*/pCA24N-yqhD. The samples were analyzed at 24 h after transferring the resting cells into the conversion medium with different concentrations of succinate. The biotransformation was conducted aerobically at 37 °C.

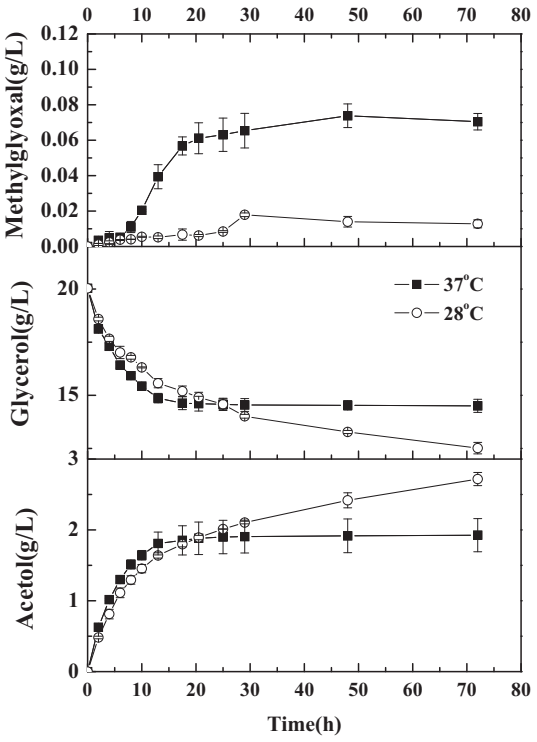

Fig. 5. Time course of the fermentation by the resting cells of *E. coli* Lin43 Δ*gloA*/pCA24N-yqhD at 28 and 37 °C. The products were detected after transferring the resting cells into the conversion medium containing 2 g/L succinate. The transformation was conducted aerobically with OD_{600} at 3.0.at 37 and 28 °C, respectively.

3.4. Effect of supplemental carbon sources on acetol production

It was reported that the specific activity of glycerol kinase in strain Lin43 increased by 6.3-fold on succinate compared to glucose (Zwaig et al., 1970). Therefore, the effect of supplemental carbon sources on acetol production was investigated. Several kinds of carbon sources (citrate, glucose, pyruvate, succinate and sucrose) were added to the conversion medium to 67 mmol C/L, respectively. As shown in Fig. 3, addition of succinate and glucose increased acetol production by 71% and 21%, respectively compared with the control, while addition of citrate and pyruvate showed slight effects on acetol production but sucrose decreased acetol production by 10%. Moreover, the yield of acetol increased by 30% when succinate was added. Furthermore, the effect of succinate concentration on acetol production was studied. As shown in Fig. 4, the highest acetol concentration was obtained with 2 g/L succinate.

3.5. Effect of temperature on MG concentration

Temperature is an important factor for biotransformations. First, we conducted the biotransformation process at 37 °C. As shown in Fig. 5, the concentration of acetol reached a maximum at 13 h and then remained unchanged. In addition, the glycerol consumption stopped and the concentration of MG reached 5.4 mM. Cells lose viability when the MG concentration in the growth medium reaches 0.6 mM (Booth et al., 2003). Therefore, these results indicate that overproduction of MG results in cell death. It was further confirmed that the cells were lost the ability to transform glycerol to acetol when they were collected and reused (data not shown). In order to alleviate the damage of MG,

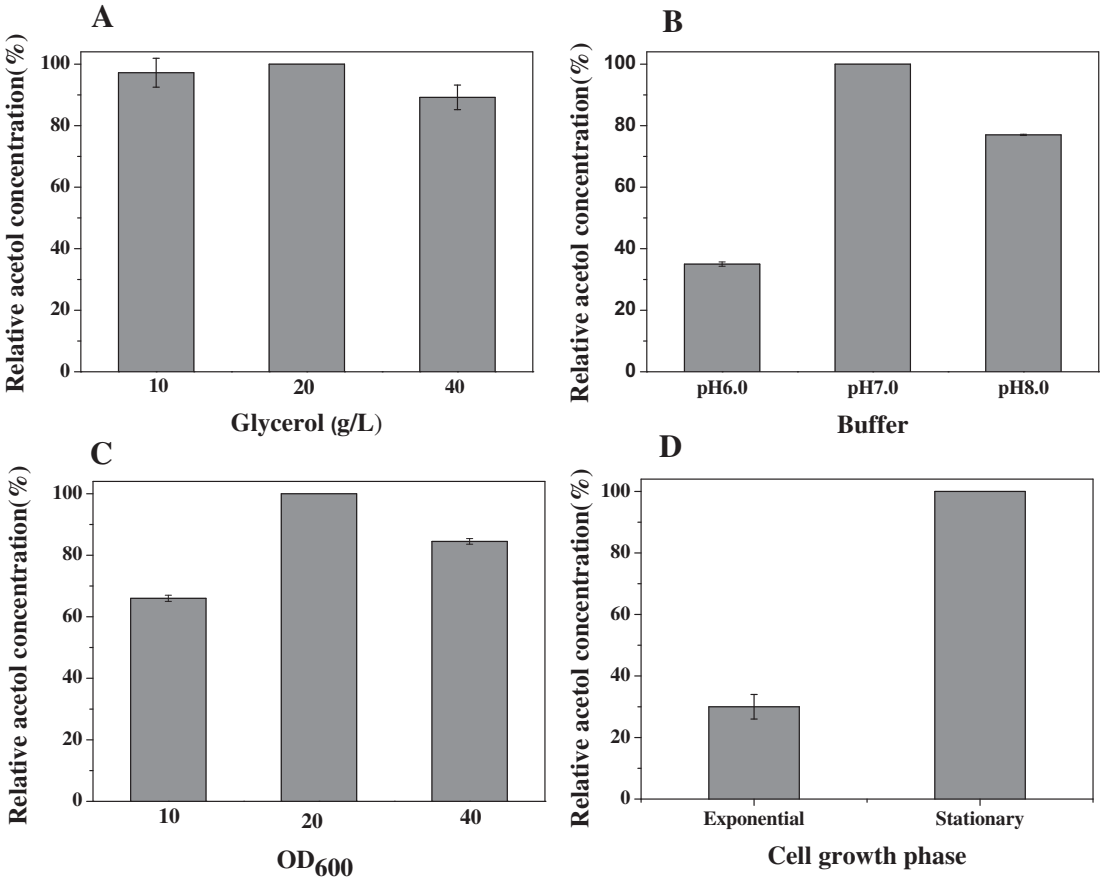

Fig. 6. Screening other parameters of the biotransformation. (A) Effect of glycerol on acetol production. (B) Effect of pH on production. (C) Effect of the cell concentration of on acetol production. (D) Effect of the cell growth phage on acetol production.

Table 2
The production of acetol by different researchers.

Strain	Carbon source	Production concentration (g/L)	Fermentation time (h)	Yield (g/g carbon source)	Refs.
Clostridium thermosaccharolyticum	Glucose	1.47	95	0.04	(Cameron and Cooney, 1986)
Saccharomy cerevisiae	Glucose	<0.1	72	<0.005	(Cameron et al., 1999)
Escherichia coli	Glucose	1.40	72	0.09	(Soucaille et al., 2008a)
Escherichia coli	Glucose	1.63	–	0.17	(Soucaille et al., 2008b)
Corynebacterium glutamicum	Glucose	3.3	84	0.055	(Niimi et al., 2011)
Escherichia coli	Glycerol	5.38	21	0.37	This study

we conducted the transformation experiments at lower temperature, 28 °C .The results shows that the resting cells produce acetol slower than those at 37 °C at the first 10 h, but the concentration of acetol increases with the incubation time. As a result, the acetol production at 28 °C is 41% higher than at 37 °C after 72 h fermentation. Moreover, the MG concentration almost remains constant at a low level at 28 °C. However, a lower productivity is obtained because it needs longer times to reach a high acetol production. Other approaches, such as NADPH regeneration and keeping a low glycerol concentration by fed batch cultivation, may be effective for the decrease of MG concentration.

3.6. Screening other basic parameters for acetol production

The toxicity of MG depends on many parameters, such as the characteristics of the growth medium and the growth phase of the cells (Ferguson et al., 1998b). In order to enhance the acetol production and alleviate the damage of MG, the effects of phosphate buffer pH, glycerol concentration, cell mass, and the cells phase on acetol production were investigated. The optimal glycerol concentration was found to be 20 g/L, as shown in Fig. 6A. Acetol production increased slightly as the glycerol concentration increased from 10 to 20 g/L and decreased at 40 g/L. As shown in Fig. 6B and C, acetol production was highest at pH 7.0 and cell OD_{600} = 20 (equal to 8.2 g cell dry weight per liter). In addition, cells from exponential and stationary phase were used for acetol production. The results showed that acetol produced by exponential cells is only 30% as those of stationary cells (Fig. 6D). Similar results were obtained when *E. coli* cells were exposed to the toxic electrophile N-ethylmaleimide (Ferguson et al., 1998a). Under the optimized conditions, we obtained 5.4 g/L acetol in 21 h at 28 °C when concentrated cell mass (OD_{600} = 20) of strain Lin43 Δ*gloA* pCA24N-yqhD was used. To our knowledge, it is the highest reported yield to date (Table 2).

4. Conclusion

Acetol is an important organic intermediate or starting material for the chemical industry. By deleting *gloA* and overexpression of *yqhD* in strain *E. coli* Lin43, here we obtained a recombinant strain that increased the acetol production and yield. Moreover, we increased the acetol production by using a resting cell system. After optimization of process conditions, this strain produced 5.4 g/L acetol. As strain Lin43 accumulates excess MG, methods to reduce the MG concentration are the key point for further improvements in acetol production by strain Lin43.

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References

Almeida, J.R., Favaro, L.C., Quirino, B., 2012. Biodiesel biorefinery: opportunities and challenges for microbial production of fuels and chemicals from glycerol waste. Biotechnol. Biofuels 5, 48–73.
Altaras, N.E., Cameron, D.C., 1999. Metabolic engineering of a 1, 2-propanediol pathway in *Escherichia coli*. Appl. Environ. Microbiol. 65, 1180–1185.
Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K.A., Tomita, M., Wanner, B.L., Mori, H., 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. 2, 1–11.
Barbelli, M.L., Santori, G.F., Nichio, N.N., 2012. Aqueous phase hydrogenolysis of glycerol to bio-propylene glycol over Pt–Sn catalysts. Bioresour. Technol. 111, 500–503.
Booth, I., Ferguson, G., Miller, S., Li, C., Gunasekera, B., Kinghorn, S., 2003. Bacterial production of methylglyoxal: a survival strategy or death by misadventure? Biochem. Soc. Trans. 31, 1406–1408.
Cameron, D.C., Cooney, C.L., 1986. A novel fermentation: the production of R (−)-1, 2-propanediol and acetol by *Clostridium thermosaccharolyticum*. Nat. Biotechnol. 4, 651–654.
Cameron, D., Shaw, A., Hoffman, M., 1999. Microbial production of hydroxyacetone and 1,2-propanediol, WO 1999/28481.
Cao, D., Fan, S.T., Chung, S.S., 1998. Identification and characterization of a novel human aldose reductase-like gene. J. Biol. Chem. 273, 11429–11435.
Chiu, C.W., Tekeei, A., Sutterlin, W.R., Ronco, J.M., Suppes, G.J., 2008. Low-pressure packed-bed gas phase conversion of glycerol to acetol. AIChE J. 54, 2456–2463.
Clomburg, J.M., Gonzalez, R., 2011. Metabolic engineering of *Escherichia coli* for the production of 1,2-propanediol from glycerol. Biotechnol. Bioeng. 108, 867–879.
Clomburg, J.M., Gonzalez, R., 2013. Anaerobic fermentation of glycerol: a platform for renewable fuels and chemicals. Trends Biotechnol. 31, 20–28.
Cooper, R., 1984. Metabolism of methylglyoxal in microorganisms. Ann. Rev. Microbiol. 38, 49–68.
Ferguson, G.P., Creighton, R.I., Nikolaev, Y., Booth, I.R., 1998a. Importance of RpoS and Dps in survival of exposure of both exponential- and stationary-phase *Escherichia coli* cells to the electrophile N-ethylmaleimide. J. Bacteriol. 180, 1030–1036.
Ferguson, G.P., Tötemeyer, S., MacLean, M., Booth, I., 1998b. Methylglyoxal production in bacteria: suicide or survival? Arch. Microbiol. 170, 209–218.
Fraval, H.N.A., McBrien, D.C.H., 1980. The effect of methyl glyoxal on cell division and the synthesis of protein and DNA in synchronous and asynchronous cultures of *Escherichia coli*. J. Gen. Microbiol. 117, 127–134.
Freedberg, W.B., Kistler, W.S., Lin, E.C., 1971. Lethal synthesis of methylglyoxal by *Escherichia coli* during unregulated glycerol metabolism. J. Bacteriol. 108, 137–144.
Jarboe, L.R., 2011. YqhD: a broad-substrate range aldehyde reductase with various applications in production of biorenewable fuels and chemicals. Appl. Microbiol. Biotechnol. 89, 249–257.
Kitagawa, M., Ara, T., Arifuzzaman, M., Ioka-Nakamichi, T., Inamoto, E., Toyonaga, H., Mori, H., 2005. Complete set of ORF clones of *Escherichia coli* ASKA library (A complete set of *E. coli* K-12 ORF archive): unique resources for biological research. DNA Res. 12, 291–299.
Ko, J., Kim, I., Yoo, S., Min, B., Kim, K., Park, C., 2005. Conversion of methylglyoxal to acetol by *Escherichia coli* aldo–keto reductases. J. Bacteriol. 187, 5782–5789.
Lee, C., Kim, I., Lee, J., Lee, K.L., Min, B., Park, C., 2010. Transcriptional activation of the aldehyde reductase YqhD by YqhC and its implication in glyoxal metabolism of *Escherichia coli* K-12. J. Bacteriol. 192, 4205–4214.
Lin, E., 1976. Glycerol dissimilation and its regulation in bacteria. Ann. Rev. Microbiol. 30, 535–578.
MacLean, M.J., Ness, L.S., Ferguson, G.P., Booth, I.R., 2002. The role of glyoxalase I in the detoxification of methylglyoxal and in the activation of the KefB K^+ efflux system in *Escherichia coli*. Mol. Microbiol. 27, 563–571.
Mohamad, M.H., Awang, R., Yunus, W.M.Z.W., 2011. A review of acetol: application and production. Am. J. Appl. Sci. 8, 1135–1139.
Niimi, S., Suzuki, N., Inui, M., Yukawa, H., 2011. Metabolic engineering of 1, 2-propanediol pathways in *Corynebacterium glutamicum*. Appl. Microbiol. Biotechnol. 90, 1721–1729.
Posada, J.A., Rincón, L.E., Cardona, C.A., 2012. Design and analysis of biorefineries based on raw glycerol: addressing the glycerol problem. Bioresour. Technol. 111, 282–293.

Sato, S., Akiyama, M., Takahashi, R., Hara, T., Inui, K., Yokota, M., 2008. Vapor-phase reaction of polyols over copper catalysts. Appl. Catal. A 347, 186–191.
Silhavy, T., Berman, M., Enquist, L., 1984. Experiments with Gene Fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Soucaille, P., Meynial-Salles, I., Voelker, F., Figge, R,. 2008a. Metabolically engineered microorganisms for production of 1,2-propanediol and acetol. WO 2008/116853.
Soucaille, P., Voelker, F., Figge, R., 2008b. Metabolically engineered microorganism useful for the production of acetol. WO 2008/116851.
Tanaka, S., Lerner, S.A., Lin, E.C., 1967. Replacement of a phosphoenolpyruvate-dependent phosphotransferase by a nicotinamide adenine dinucleotide-linked dehydrogenase for the utilization of mannitol. J. Bacteriol. 93, 642–648.
Wang, Z., Yang, S.-T., 2013. Propionic acid production in glycerol/glucose co-fermentation by *Propionibacterium freudenreichii* subsp. shermanii. Bioresour. Technol. 137, 116–123.
Yamaguchi, A., Hiyoshi, N., Sato, O., Shirai, M., 2010. Dehydration of triol compounds in high-temperature liquid water under high-pressure carbon dioxide. Top. Catal. 53, 487–491.
Yang, F., Hanna, M., Sun, R., 2012. Value-added uses for crude glycerol–a byproduct of biodiesel production. Biotechnol. Biofuels 5, 13–22.
Yazdani, S.S., Gonzalez, R., 2007. Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. Curr. Opin. Biotechnol. 18, 213–219.
Zwaig, N., Kistler, W.S., Lin, E.C., 1970. Glycerol kinase, the pacemaker for the dissimilation of glycerol in *Escherichia coli*. J. Bacteriol. 102, 753–759.