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ERRATUM

Erratum to: Deletion of *arcA* increased the production of acetyl-CoA-derived chemicals in recombinant *Escherichia coli*

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Unfortunately, one of the corresponding author "Guang Zhao" name has been published incorrectly in the original article as "Gao Zhao". It has been corrected in this erratum.

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ORIGINAL RESEARCH PAPER

Deletion of *arcA* increased the production of acetyl-CoA-derived chemicals in recombinant *Escherichia coli*

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Abstract
Objective Acetyl-CoA is used to produce many valuable metabolites in *Escherichia coli*. However, acetate overflow is a major shortcoming. Knockout of the global regulator gene, *arcA,* may solve this problem.
Results The *arcA* gene of *E. coli* BL21(DE3) was knocked out, and the production of phloroglucinol (PG) and 3-hydroxypropionate (3HP), both derived from acetyl-CoA, were used to evaluate its effect. The *arcA* mutants had higher cell yields and higher glucose utilization efficiencies than the corresponding control strains, and the productions of PG and 3HP were 0.92 g/l and 0.27 g/l, respectively; more than twice that of the control strains. Furthermore, *arcA* knockout also showed significant repression on formation of acetate, the major byproduct in fermentation. Acetate concentrations were decreased 69.4 % and 87 % by *arcA* knockout during the production of PG and 3HP, respectively.
Conclusions The *arcA* gene knockout is a solution to acetate overflow and may improve production of a wide range of acetyl-CoA-derived metabolites.

Keywords Acetate-derived metabolites · Acetyl-CoA · *arcA* · *Escherichia coli* · 3-Hydroxypropionate · Phloroglucinol

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Introduction

Acetyl-CoA is a common metabolic intermediate in microorganisms and is the precursor of large number of valuable metabolites in recombinant *Escherichia coli* strains, including polyhydroxyalkanoates, isoprenoids, polyketides, lipids and butyrate (Chen et al. 2013). However, acetate accumulation, also known as "acetate overflow", is a major drawback to production of acetyl-CoA-derived chemicals in *E. coli*. Acetate overflow, in general, is caused by an imbalance between the pathways of Embden–Meyerhof–Parnas (EMP) and tricarboxylic acid (TCA) cycle in rapidly growing cells (Farmer and Liao 1997; Wong et al. 2008). Many approaches have been put forward to decrease acetate secretion. Knockout of a list of genes related to acetate accumulation decreased acetate secretion to 10 % of a wild strain (Kang et al. 2009). Thus, cells can decrease acetate accumulation and

increase production of acetyl-CoA-derived chemicals in three ways: (1) enhancing the activity of the TCA cycle, (2) repressing glucose consumption rate or the cell growth rate, (3) deleting specific genes related to acetate accumulation.

ArcA is an important global regulator in *E. coli* and phosphorylated ArcA represses expression of genes of the TCA cycle and the glyoxylate shunt, such as *gltA, acnA*, *icdA*, *sucABCD*, *sdhCDAB*, *fumAC*, *mdh* and *aceBAK* (Lynch and Lin 1996; Perrenoud and Sauer 2005). Knockout of *arcA* alleviates the repression of TCA cycle and enhances the heterologous genes expression (Vemuri et al. 2006). Acetate excretion was significantly decreased and the production of D-lactate was increased by deleting *arcA* (Kim et al. 2013; Nikel et al. 2008). It is, therefore, plausible to enhance production of chemicals derived from acetyl-CoA by *arcA* deletion.

The biosynthetic pathways of two acetyl-CoA-derived chemicals, phloroglucinol (PG) and 3-hydroxypropionate (3HP) which are both important bulk chemicals for a wide range of industrial applications (Rathnasingh et al. 2009; Singh et al. 2009), have been constructed in recombinant *E. coli*, respectively (Fig. 1) (Cao et al. 2011; Liu et al. 2013). The engineered strain Q1944 (*E. coli* BL21(DE3)/pA-*accADBC*/pET-*phlDmar*) accumulated 0.51 g PG/l, and 0.15 g 3HP/l was produced by the strain Q1319 (*E. coli* BL21(DE3)/pA-*accADBC*/pMCR-N–C) after cultivation in M9 salts medium under shake-flask conditions. Unfortunately, this is still too low and not economically feasible for industrial applications.

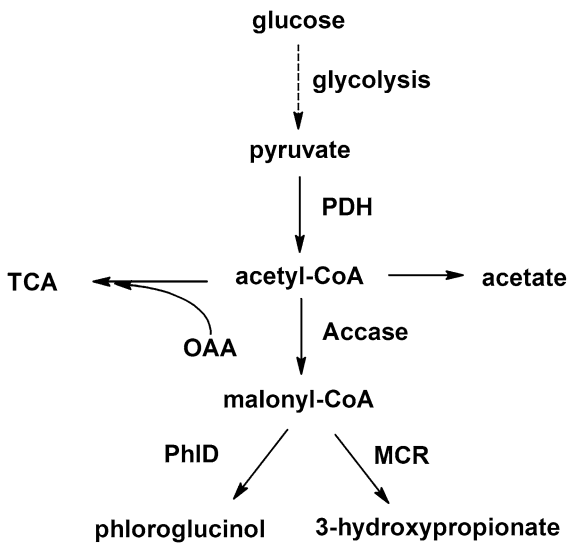

Fig. 1 Biosynthetic pathways of PG and 3HP with glucose as the sole carbon source. *PDH* pyruvate dehydrogenase complex; *ACCase* acetyl-CoA carboxylase; *PhlD* polyketide synthase; *MCR* malonyl-coenzyme A reductase; *OAA* oxaloacetic acid

To improve the production of acetyl-CoA-derived chemicals, the *arcA* gene of *E.coli* BL21(DE3) was knocked out, and the resultant strain was used to produce PG and 3HP. The effects of *arcA* deletion on cell growth and metabolism were determined and discussed.

Materials and methods

Bacterial strains and growth conditions

Escherichia coli χ7213 strain was used as the host to prepare the suicide vector. *Escherichia coli* strains were cultivated at 37 °C in lysogeny broth (LB) unless specified. Diaminopimelic acid (DAP) (50 mg/ml) was needed for the growth of *E. coli* χ7213 strain. When necessary, 100 μg ampicillin/ml and 50 μg chloramphenicol/ml were added to the medium. LB agar containing 10 % (w/v) sucrose was used for *sacB* gene-based counter selection in allelic exchange experiments.

Strains construction

The *arcA* mutant was constructed using suicide vector pRE112 as previously described (Edwards and Schifferli 1998). Two pairs of primers,754 (5′-CGGGGTACC GCTGGCGCTTTCTGAAC-3′)/755(5′-GTGAACTG CGCGAGCAGGCTGAAAACCCAGGCAAAATT C-3′) and 756 (5′-GCCTGCTCGCGCAGTTCACG-3′)/757(5′-CGAGCTCCGTTTATTAGTTGTATGA TGC-3′) were used to amplify fragments upstream and downstream of *arcA* from BL21(DE3) chromosome, respectively. This two fragments were ligated by overlapping PCR, and the PCR product was cloned into pRE112 vector between the *Kpn*I and *Sac*I sites to generate plasmid pRE112-△*arcA*. The *arcA* mutant was introduced into BL21(DE3) strain by allelic exchange using pRE112-△*arcA*, and the resultant strain was named as Q1949. The four subunits of *accA* (GeneID: 6062185), *accD* (GeneID: 6059083) and *accBC* (GeneID: 6058890 and 6058863) were cloned into pACYCDuet-1, resulting

in plasmid pA-*accADBC,* the *phlD* gene (GenBank accession no. EU554263) and *mar* gene (GeneID: 6060688) were cloned into pET30a, resulting in plasmid pET-*phlDmar* (Cao et al. 2011). The plasmids pA-*accADBC* and pET-*phlDmar* were transformed into Q1949 to generate Q1963. The *mcr*-N and *mcr*-C (GenBank accession no. YP_001636209) were cloned into pETDuet-1, resulting in plasmid pMCR-N–C (Liu et al. 2013). The plasmids pA-*accADBC* and pMCR-N–C were transformed into Q1949 to generate Q2373.

Shake-flask cultivation of the recombinant *E.coli* strains

Shake-flask experiments were carried out in triplicate in 250 ml flasks containing 50 ml fermentation medium. The fermentation medium for PG production contains 9.8 g $K_2HPO_4{\cdot}3H_2O$/l, 2.1 g citric acid$\cdot H_2O$/l, 0.3 g ferric ammonium citrate/l, 3.0 g $(NH_4)_2SO_4$/l, 0.2 g $MgSO_4{\cdot}7H_2O$/l, 20 g glucose/l and trace metal [3.7 g $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$/l, 2.9 g $ZnSO_4{\cdot}7H_2O$/l, 24.7 g H_3BO_3/l, 2.5 g $CuSO_4{\cdot}5H_2O$/l, 15.8 g $MnCl_2{\cdot}4H_2O$/l]. The fermentation medium for 3HP production contained 14 g $K_2HPO4{\cdot}3H_2O$/l, 5.2 g KH_2PO_4/l, 1 g NaCl/l, 1 g NH_4Cl/l, 0.25 g $MgSO_4{\cdot}7H_2O$/l, 0.2 g yeast extract/l, 20 g glucose/l. The pH of minimal medium was adjusted to 7.0 by adding NH_4OH. The strains were grown overnight at 37 °C with shaking in LB medium and then 1:100 diluted into 50 ml fermentation medium. IPTG was added to 0.1 mM at ~0.8 OD_{600} and cultures were further incubated at 30 °C. Ten milligram biotin/l and 20 mM $NaHCO_3$/l were added to the culture 2 h later for induction for 3HP production.

Analytical methods

The cell concentration was measured from the OD_{600} value (1 OD unit = 0.36 g CDW/l). The concentration of residual glucose was quantified by using an SBA-40D biological sensing analyzer. The concentration of PG in the fermentation supernatant was quantified from the A_{446} value using the colorimetric reaction at between cinnamaldehyde and PG (Zha et al. 2008). 3HP concentration in the medium was determined by HPLC as described previously (Rathnasingh et al. 2012).

Results and discussion

Effects of *arcA* gene knockout on cell growth

The *arcA* gene in PG- and 3HP-producing strains was knocked out by allelic exchange using suicide vector pRE112. Cell growth and glucose consumption of the control strains (Q1944 and Q1319) and *arcA* mutants (Q1963 and Q2373) were monitored during their cultivation. As shown in Fig. 2, the *arcA* mutants had a lower growth rate than their corresponding control strains. After cultivation, the cell dry weight (CDW) of control strains Q1944 and Q1319 were 2.05 ± 0.06 and 3.08 ± 0.12 g/l, while the *arcA* mutants Q1963 and Q2373 were 1.9 ± 0.1 and 2.45 ± 0.13 g CDW/l, respectively (Table 1). Consistent with the growth status, the *arcA* mutants consumed glucose more slowly than the control strains (Fig. 2 and Table 1).

The cell yield of each strain was also calculated. *arcA* mutant Q1963 achieved 0.14 ± 0.01 g CDW/g glucose, 1.17-fold higher than control strain Q1944, and a similar result was obtained with the strains Q2373 and Q1319 (Table 1), suggesting that knockout of *arcA* makes cells produce more biomass than the control strain with consumption of the same amount of glucose thereby and improving glucose utilization efficiency. Although the reason still remains unclear, the *arcA* mutant can be used in industrial fermentation to reduce production costs.

Knockout of *arcA* increases PG and 3HP production

The effect of *arcA* knockout on PG and 3HP production was determined (Table 1). The *arcA* deletion enhanced PG production from 0.41 ± 0.02 g/l to 0.92 ± 0.02 g/l, showing a 2.25-fold increase. The glucose conversion efficiency of Q1963 was 6.67 %, 2.74-time higher than that of control strain Q1944. For 3HP production, the *arcA* mutant Q2373 accumulated 0.27 g 3HP/l after 48-h cultivation, while 0.13 ± 0.01 g 3HP/l was produced by the control strain Q1319. The conversion efficiency of glucose to 3HP was also increased to 2.2 % by *arcA* deletion.

The improvement in PG and 3HP production could be caused by two reasons. Firstly, the *arcA* mutant could improve the TCA cycle flux and expression of heterologous genes (Nizam et al. 2009; Perrenoud and

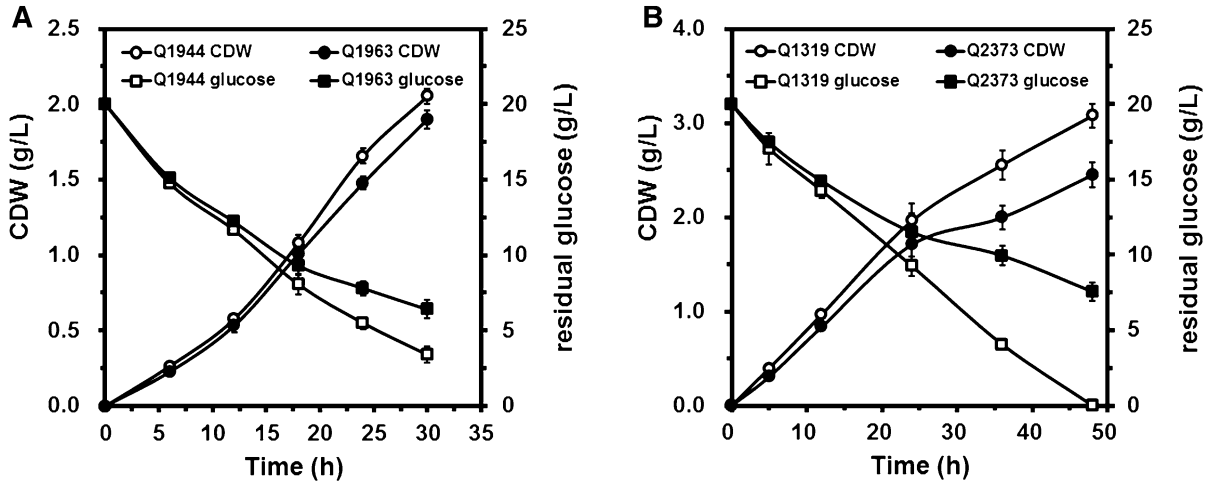

Fig. 2 Cell growth and glucose consumption of different strains when cultivated in shaking flasks. **a** The cell growth of PG-producing strains Q1944 and Q1963 and concentration of residual glucose in their cultures. **b** The cell growth of 3HP-producing strains Q1319 and Q2373 and concentration of residual glucose in their cultures

Table 1 Effects of *arcA* knockout on growth of cells and production of acetyl-CoA-derived metabolites

	Phloroglucinol		3-Hydroxypropionate	
	Q1944*	Q1963**	Q1319*	Q2373**
CDW (g/l)	2.05 ± 0.06	1.9 ± 0.1	3.08 ± 0.12	2.45 ± 0.13
Residual glucose (g/l)	3.4 ± 0.28	6.4 ± 0.59	0	7.55 ± 0.64
Glucose consumption rate (mmol/g CDW/h)	1.5 ± 0.1	1.33 ± 0.12	0.75 ± 0.03	0.59 ± 0.02
Cell yield (g CDW/g Glu)	0.12 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.2 ± 0.01
Metabolite production (g/l)	0.41 ± 0.02	0.92 ± 0.02	0.13 ± 0.01	0.27 ± 0.03
Glucose conversion (%)	2.47	6.67	0.65	2.17
Acetate concentration (g/l)	3.37 ± 0.08	1.03 ± 0.13	5.47 ± 0.13	0.71 ± 0.12
Acetate production rate (mmol/g CDW/h)	0.91 ± 0.06	0.3 ± 0.03	0.62 ± 0.03	0.1 ± 0.01

Experiments were conducted in triplicate, and date represents mean ± standard deviation

* Control strains

** *arcA* knock-outs

Sauer 2005). Secondly, the flux through pyruvate dehydrogenase (PDH) complex, which catalyzes pyruvate to acetyl-CoA, in the *arcA* mutant was 18 % higher than that in control strain (Nizam et al. 2009), resulting in its flux to PG and 3HP.

Deletion of *arcA* gene overcomes acetate overflow

The effect of *arcA* knockout on production of acetate was also investigated (Table 1). After 30 h cultivation, the PG-producing strains Q1944 and Q1963 produced 3.37 ± 0.08 and 1.03 ± 0.13 g acetate/l, respectively, indicating that *arcA* mutant could significantly decrease the acetate accumulation during production of acetyl-CoA-derived chemicals. The similar result was also observed in 3HP fermentation. The *arcA* mutant Q2373 produced only 0.71 ± 0.12 g acetate/l, which was decreased by 87 % when compared with control strain Q1319. This decrease of acetate formation could be a result of a higher TCA cycle flux and lower glucose consumption rate.

The effect of *arcA* knockout on acetate accumulation in *E. coli* K12 strain, as studied by Nizam et al. 2009, was not as significant as shown here. This could

be caused by the host strain. Strain BL21 has a lower catabolite repression than K12 strain, and can consume acetate through the acetyl-CoA synthetase (Acs) pathway in the presence of glucose (Waegeman et al. 2012). In our study, deletion of *arcA* in BL21 strain could enhance the activity of TCA cycle and the glyoxylate shunt, and more acetate was assimilated, resulting in improved production of acetyl-CoA derived chemicals and decreased formation of the major byproduct, acetate.

Conclusions

A platform cell factory was constructed to enhance the acetyl-CoA-derived metabolites by deleting *arcA* in *E. coli* BL21(DE3) strain. The *arcA* mutants had higher cell yields and higher glucose utilization efficiencies than the control strains. The production of PG and 3HP was improved by 2.25-fold and 2.08-fold, respectively. Knockout of *arcA* also exhibited surprising efficacy of overcoming acetate formation. It is believed that this genetic modification would be a useful tool to improve production of a wide range of acetyl-CoA-derived chemicals.

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