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# Engineered Synthetic Pathway for Isopropanol Production in *Escherichia coli*▽†

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**A synthetic pathway was engineered in *Escherichia coli* to produce isopropanol by expressing various combinations of genes from *Clostridium acetobutylicum* ATCC 824, *E. coli* K-12 MG1655, *Clostridium beijerinckii* NRRL B593, and *Thermoanaerobacter brockii* HTD4. The strain with the combination of *C. acetobutylicum thl* (acetyl-coenzyme A [CoA] acetyltransferase), *E. coli atoAD* (acetoacetyl-CoA transferase), *C. acetobutylicum adc* (acetoacetate decarboxylase), and *C. beijerinckii adh* (secondary alcohol dehydrogenase) achieved the highest titer. This strain produced 81.6 mM isopropanol in shake flasks with a yield of 43.5% (mol/mol) in the production phase. To our knowledge, this work is the first to produce isopropanol in *E. coli*, and the titer exceeded that from the native producers.**

The search for feasible petroleum substitutes (bioalcohols, biodiesel, and bioplastics) from renewable resources has recently become a global priority as the atmospheric carbon dioxide level rises and petroleum resources become increasingly expensive. Isopropanol is one of the secondary alcohols that can be produced by microbes (17). It could be used as a biofuel to partially replace gasoline. Isopropanol is also used instead of methanol to esterify fat and oil to produce biodiesel, which reduces its tendency to crystallize at low temperatures (11). Finally, isopropanol can be dehydrated to yield propylene (9), which is currently derived from petroleum as a monomer for making polypropylene.

Several species of *Clostridium* have been evaluated for isopropanol production, including 52 strains of *Clostridium beijerinckii* (4). The maximum production of isopropanol from these strains was 30 mM. Limited knowledge about metabolic regulation of the strains and the difficulty of gene manipulation have hindered further improvements in isopropanol production. On the other hand, *Escherichia coli* is one of most studied and easily manipulated organisms for metabolic engineering (2, 5, 15). The bacterium has already been shown to produce high titers of ethanol (6) and many other biochemicals (1, 2, 5, 12, 22).

Bermejo et al. (1) produced acetone in *E. coli* by introducing four genes from *Clostridium acetobutylicum* ATCC 824 (*thl*, *ctfAB*, and *adc* encoding acetyl-coenzyme A [CoA] acetyltransferase, acetoacetyl-CoA transferase, and acetoacetate decarboxylase, respectively) under the control of the *thl* promoter from *C. acetobutylicum* (1). This engineered *E. coli* strain produced almost the same level of acetone as *C. acetobutylicum* ATCC 824 does. However, isopropanol production in *E.coli* has not been reported. Here, we engineered a synthetic pathway for the production of isopropanol in *E. coli*.

The strategy for the biosynthesis of isopropanol in *E. coli* utilizes the pathway modeled after *C. beijerinckii*, which produces isopropanol from acetyl-CoA via acetone (Fig. 1). First, an acetyl-CoA acetyltransferase condenses two molecules of acetyl-CoA to one molecule of acetoacetyl-CoA (23). Next, an acetoacetyl-CoA transferase transfers CoA from acetoacetyl-CoA to acetate or to butyrate (24), forming acetoacetate, which is then converted to acetone and $CO_2$ by an acetoacetate decarboxylase (19). Finally, a primary-secondary alcohol dehydrogenase (hereafter referred to as the secondary alcohol dehydrogenase [SADH]) converts acetone to isopropanol in an NADPH-dependent reaction (3). To optimize the pathway, we also evaluated the native *E. coli* acetyl-CoA acetyltransferase (encoded by *atoB*) and acetoacetyl-CoA transferase (encoded by *atoAD*) (8) in performing the initial steps of acetone production. Furthermore, we compared the activities of the SADHs (encoded by *adh*) from *C. beijerinckii* NRRL B593 (7) and *Thermoanaerobacter brockii* HTD4 (10) in performing the final step of isopropanol production in *E. coli*. These two SADHs have previously been expressed and characterized in *E. coli* (18).

## MATERIALS AND METHODS

**Strains and plasmid construction.** Table 1 shows the strains and plasmids used in this study. *E. coli* strain B (ATCC 11303) with *lacI*$^q$ introduced from *E. coli* DH5αZ1 (14) by P1 transduction was used as the host strain and designated TA11. Multiple combinations of the acetone pathway genes *thl*, *ctfAB*, *atoB*, *atoAD*, and *adc* were cloned into a ColE1-delivered plasmid (pSA40) under the control of the $P_L lacO_1$ promoter (14). The resulting plasmids were named pTA29 (*atoB ctfAB adc*), pTA30 (*atoB atoAD adc*), pTA39 (*thl atoAD adc*), and pTA41 (*thl ctfAB adc*) (Table 1 shows the details). The *adh* gene from *C. beijerinckii* or *T. brockii* was cloned into a p15A-delivered vector (pZA31-luc) under the control of $P_L lacO_1$ to generate plasmids pTA36 and pTA18, respectively. Each combination of the acetone pathway genes and the isopropanol-producing genes was introduced into TA11 for evaluation.

The same ribosome binding site (RBS) (GAAGGAGATATACAT) was used for all genes of the isopropanol production pathway except the *atoB* and *thl* genes, which were cloned downstream of the $P_L lacO_1$ promoter using the expression construct's RBS. The source of the RBS used was the pET-31b(+) plasmid (Invitrogen, Carlsbad, CA). All genes except the two *adh* genes were PCR amplified from chromosomal DNA and their sequences were verified. The two *adh* genes were synthesized by Epoch Biolabs (Houston, TX) after codon optimization for *E. coli*. The following conditions were used for optimization. A

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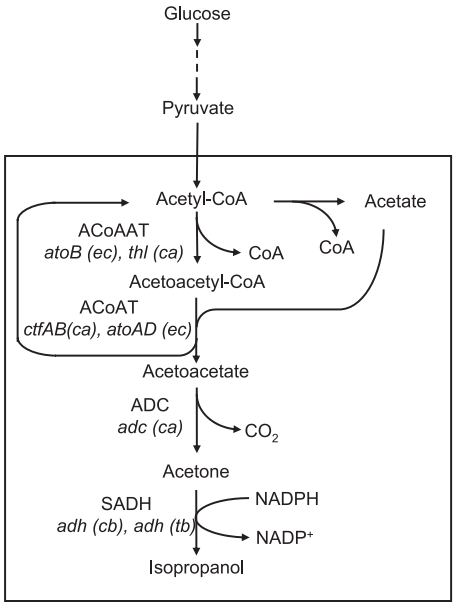



FIG. 1. The metabolic pathway for isopropanol production. The box shows the synthetic pathway for isopropanol production from acetyl-CoA engineered in this study. The dashed line indicates omitted steps. ACoAAT, acetyl-CoA acetyltransferase (encoded by *E. coli atoB* or *C. acetobutylicum thl*); ACoAT, acetoacetyl-CoA transferase (encoded by *C. acetobutylicum ctfAB* or *E. coli atoAD*); ADC, acetoacetate decarboxylase (encoded by *C. acetobutylicum adc*); SADH, encoded by *C. beijerinckii adh* or *T. brockii adh*. *ec*, *E. coli*; *ca*,*C. acetobutylicum*; *cb*, *C. beijerinckii*; *tb*, *T. brockii*.

15% cutoff was used for codon efficiency; any codon below 15% was removed, except for positions with strong secondary structures (in which case codons of lower frequency were used to alleviate the problem). Secondary structure was checked using a built-in M-fold module. Stem-loop and pseudo-Shine-Dalgarno sequences were avoided. The number of base pairs changed by this optimization were about 290 for *C. beijerinckii* and 240 for *T. brockii*. Restriction enzymes, phosphatase (New England Biolabs, Ipswich, MA), ligase (rapid DNA ligation kit; Roche, Mannheim, Germany), and DNA polymerase (KOD DNA polymerase; EMD Chemicals, San Diego, CA) were used for cloning. Oligonucleotides were synthesized by Invitrogen (Carlsbad, CA).

**pTA29 ($P_L$lacO$_1$::*atoB ctfAB adc*).** To replace $P_L$tetO$_1$ of pZE21-MCS1 (14) with $P_L$lacO$_1$, pZE12-luc was digested with AatII and Acc65I. The shorter fragment was purified and cloned into plasmid pZE21-MCS1 cut with the same enzymes, creating pSA40. To clone *atoB*, *ctfAB*, and *adc*, Acc65I-SalI (*atoB*), SalI-XmaI (*ctfAB*), and XmaI-BamHI (*adc*) recognition sites in pSA40 were used. AatII and AvrII recognition sites were used to replace the kanamycin resistance gene with the ampicillin resistance gene. To clone *atoB*, genomic DNA of *E. coli* K-12 MG1655 was used as a template with a pair of primers, TA15F (5′ CGCGGTACCATGAAAAATTGTGTCATCGTCAGTG 3′) and TA16R (5′ CCGCGTCGACTTAATTCAACCGTTCAATCACCATC 3′), and the PCR products were digested with Acc65I and SalI. To clone *ctfAB*, genomic DNA of *C. acetobutylicum* ATCC 824 (ATCC) was used as a template with a pair of primers, TA19F (5′ CCGCGTCGACGAAGGAGATATACATATGAACTCT AAAATAATTAGATTTG 3′) and TA4R (5′ CGCCCCGGGCTAAACAGCC ATGGGTCTAAGTTCA 3′), and the PCR products were digested with SalI and XmaI. To clone *adc*, genomic DNA of *C. acetobutylicum* ATCC 824 was used as a template with a pair of primers, TA20F (5′ CGCCCCGGGGAAGGAGATA TACATATGTTAAAGGATGAAGTAATTAAAC 3′) and TA6R (5′ CGCGG ATCCTTACTTAAGATAATCATATATAACT 3′), and the PCR products were digested with XmaI and BamHI.

**pTA30 ($P_L$lacO$_1$::*atoB atoAD adc*).** To clone *atoAD*, genomic DNA of *E. coli* K-12 MG1655 was used as a template with a pair of primers, TA21F (5′ CCG CGTCGACGAAGGAGATATACATATGAAAACAAAATTGATGACA TTAC 3′) and TA18R (5′ CGCCCCGGGTCATAAATCACCCCGTTGCGT ATTC 3′). The PCR products were digested with SalI and XmaI and cloned into plasmid pTA29 cut with the same enzymes.

**pTA39 ($P_L$lacO$_1$::*thl atoAD adc*).** To clone *thl*, genomic DNA of *C. acetobutylicum* ATCC 824 was used as a template with a pair of primers, TA13F (5′ CGCGGTACCATGAAAGAAGTTGTAATAGCTAGTG 3′) and TA14R (5′ CCGCGTCGACCTAGCACTTTTCTAGCAATATTGC 3′). The PCR products were digested with Acc65I and SalI and cloned into plasmid pTA30 cut with the same enzymes.

**pTA41 ($P_L$lacO$_1$::*thl ctfAB adc*).** To clone *thl*, genomic DNA of *C. acetobutylicum* ATCC 824 was used as a template with a pair of primers, TA13F and TA14R. The PCR products were digested with Acc65I and SalI and cloned into plasmid pTA29 cut with the same enzymes.

**pTA18 ($P_L$lacO$_1$::*adh* [*T. brockii*]).** To clone *adh* (*T. brockii*), synthesized DNA of *T. brockii* HTD4 (Epoch Biolabs) was used as a template with a pair of primers, TA7F (5′ CGCGGTACCATGAAAGGTTTTGCAATGCTGTCCA 3′) and TA8R (5′ CGCTCTAGACTATGCTAAAATCACCACTGGTTT AATT 3′). The PCR products were digested with Acc65I and XbaI and cloned into plasmid pZE12-luc cut with the same enzymes, creating pTA8. To replace the replication origin and the ampicillin resistance gene with p15A and the kanamycin resistance gene, pZA31-luc(14) was digested with AatII and AvrII. The shorter fragment was purified and cloned into pTA8 cut with the same enzymes to create pTA18. The codon usage of the synthesized DNA was optimized for expression in *E. coli*, and stem-loop structures were avoided by checking with a secondary-structure prediction program. This sequence is shown in the supplemental material.

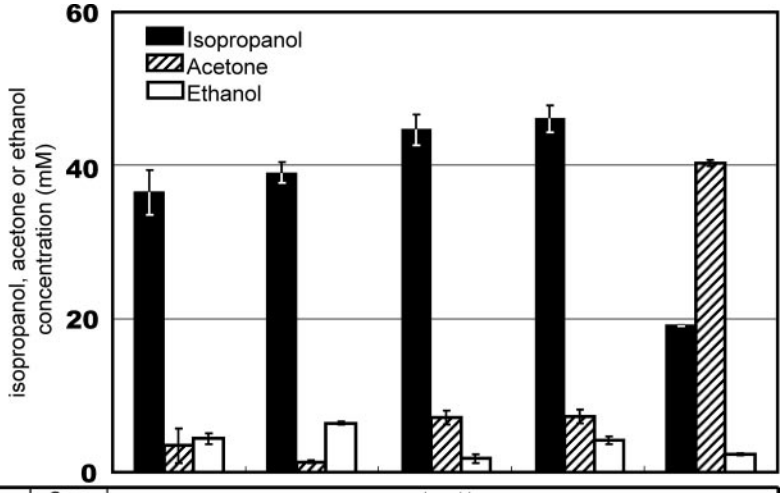
**pTA36 ($P_L$lacO$_1$::*adh* [*C. beijerinckii*]).** To clone *adh* (*C. beijerinckii*), the plasmid with synthesized DNA of *C. beijerinckii* NRRL B593 (Epoch Biolabs) was digested with Acc65I and SalI and cloned into plasmid pZE12-luc cut with the same enzymes, creating pTA34. To replace the replication origin and the ampicillin resistance gene with p15A and the kanamycin resistance gene, pZA31-luc was digested with AatII and AvrII. The shorter fragment was purified and cloned into the plasmid cut with the same enzymes to create pTA36. The codon usage of the synthesized DNA was optimized in the same way as the *adh* gene from *T. brockii*. This sequence is shown in the supplemental material.

**Medium and cultivation.** As the preculture medium, SD-7 containing 2% glucose was prepared ($NH_4Cl$, 7.0 g/liter; $KH_2PO_4$, 1.5 g/liter; $Na_2HPO_4$, 1.5 g/liter; $K_2SO_4$, 0.35 g/liter; $MgSO_4 \cdot 7H_2O$, 0.17 g/liter; trace elements, 0.8 ml/liter; yeast extract, 5 g/liter) as described previously (13). SD-8 medium ($NH_4Cl$, 7.0 g/liter; $KH_2PO_4$, 7.5 g/liter; $Na_2HPO_4$, 7.5 g/liter; $K_2SO_4$, 0.85 g/liter; $MgSO_4 \cdot 7H_2O$, 0.17 g/liter; trace elements, 0.8 ml/liter; yeast extract; 10 g/liter) (13) containing 2% glucose was used for fermentations. The trace element solution contained the following (in grams per liter of 5 M HCl): $FeSO_4 \cdot 7H_2O$, 40.0; $MnSO_4 \cdot H_2O$, 10.0; $Al_2(SO4)_3$, 28.3; $CoCl_2 \cdot 6H_2O$, 4.0; $ZnSO_4 \cdot 7H_2O$, 2.0; $Na_2MoO_4 \cdot 2H_2O$, 2.0; $CuCl_2 \cdot 2H_2O$, 1.0; and $H_3BO_4$, 0.5. Antibiotics were added as appropriate (ampicillin, 100 μg/ml, and chloramphenicol, 40 μg/ml).

Preculture with 5 ml of SD-7 medium in a test tube was performed at 37°C overnight (17 h) on a rotary shaker (250 rpm); 250 μl of overnight culture was inoculated into 25 ml of SD-8 medium in a 250-ml flask with baffles. When the optical density at 600 nm was ~1.0 (3 h), 0.1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) was added for induction. Cells were grown at 37°C for 30.5 h on a rotary shaker (250 rpm) and harvested at 0, 3, 6.5, 9.5, 12, 24, 28.5, and 30.5 h.

TABLE 1. Strains and plasmids used

| *E. coli* strain or plasmid | Relevant characteristics | Source or reference |
|---|---|---|
| *E. coli* strains | | |
| ATCC 11303 | Wild type | ATCC |
| TA11 | Same as ATCC 11303, but *lac* $I^q$ $Tet^r$ | This study |
| Plasmids | | |
| pZE12-luc | $P_L$lacO$_1$::*luc* ColE1 $Amp^r$ | 14 |
| pZE21-MCS1 | $P_L$tetO$_1$::MCS1 ColE1 $Kan^r$ | 14 |
| pZA31-luc | $P_L$tetO$_1$::*luc* p15A $Cm^r$ | 14 |
| pSA40 | $P_L$lacO$_1$::MCS1 ColE1 $Kan^r$ | This study |
| pTA29 | $P_L$lacO$_1$::*atoB ctfAB adc* ColE1 $Amp^r$ | This study |
| pTA30 | $P_L$lacO$_1$::*atoB atoAD adc* ColE1 $Amp^r$ | This study |
| pTA39 | $P_L$lacO$_1$::*thl atoAD adc* ColE1 $Amp^r$ | This study |
| pTA41 | $P_L$lacO$_1$::*thl ctfAB adc* ColE1 $Amp^r$ | This study |
| pTA18 | $P_L$lacO$_1$::*adh* (*T. brockii*) p15A $Cm^r$ | This study |
| pTA8 | $P_L$lacO$_1$::*adh* (*T. brockii*) ColE1 $Amp^r$ | This study |
| pTA36 | $P_L$lacO$_1$::*adh* (*C. beijerinckii*) p15A $Cm^r$ | This study |
| pTA34 | $P_L$lacO$_1$::*adh* (*C. beijerinckii*) ColE1 $Amp^r$ | This study |




| Enzyme | Gene name | plasmid | | | | |
|---|---|---|---|---|---|---|
| | | pTA41/pTA36 | pTA29/pTA36 | pTA30/pTA36 | pTA39/pTA36 | pTA39/pTA18 |
| ACoAAT | *thl(ca)* | + | | | + | + |
| | *atoB(ec)* | | + | + | | |
| ACoAT | *ctfAB(ca)* | + | + | | | |
| | *atoAD(ec)* | | | + | + | + |
| ADC | *adc(ca)* | + | + | + | + | + |
| SADH | *adh (cb)* | + | + | + | + | |
| | *adh (tb)* | | | | | + |

FIG. 2. Comparison of maximum isopropanol production levels by each combination of the pathway genes. ACoAAT, acetyl-CoA acetyltransferase; ACoAT, acetoacetyl-CoA transferase; ADC, acetoacetate decarboxylase; *ec*, *E. coli*; *ca*, *C. acetobutylicum*; *cb*, *C. beijerinckii*; *tb*, *T. brockii*. The error bars indicate standard deviations of at least two independent experiments.

**Glucose and fermentation product analysis.** Glucose was measured using a glucose analysis reagent (Sigma Aldrich). Various alcohols were quantified by gas chromatography with a flame ionization detector. The reported analysis conditions (21) were modified for this study. The system consisted of a model 5890A gas chromatograph (Hewlett-Packard, Avondale, PA) and a model 7673A automatic injector, sampler, and controller (Hewlett-Packard). The separation of alcohol compounds was carried out with a DB-WAX capillary column (30 m; 0.32-mm inside diameter; 0.50-μm film thickness; Agilent Technologies). The gas chromatograph oven temperature was initially held at 40°C for 5 min and raised with a gradient of 15°C/min to 120°C, followed by a gradient of 50°C/min to 230°C, where it was held for 4 min. Helium was used as the carrier gas, with 9.3-lb/in$^2$ inlet pressure. The injector and detector were maintained at 225°C; 0.5 μl supernatant of the culture broth was injected in split-injection mode (1:15 split ratio). 1-Propanol was used as the internal standard.

For other secreted metabolites, filtered supernatant (20 μl) was applied to an Agilent 1100 high-performance liquid chromatograph equipped with an autosampler (Agilent Technologies) and a Bio-Rad Aminex HPX87 column (5 mM $H_2SO_4$; 0.6 ml/min; column temperature, 65°C; Bio-Rad Laboratories, Hercules, CA) (16). Organic acids (fumarate, lactate, citrate, pyruvate, formate, malate, acetate, and succinate) were detected using a photodiode array detector at 210 nm.

**Secondary alcohol dehydrogenase enzyme assay.** Cells harvested 4 h after induction were used for enzyme assays after centrifugation. Crude extract in 50 mM Tris-chloride (pH 8) was prepared under anaerobic conditions with 0.1-mm glass beads and a Mini Bead Beater 8 (Biospec Products, Oklahoma). The assay was carried out according to the method described previously (7). SADH activities were measured by following the reduction of acetone (6.7 mM) with NADPH at 25°C under Ar. The assay mixture contained 50 mM Tris-Cl buffer (pH 7.5), 1 mM dithiothreitol, and 0.2 mM NADPH. One unit of activity is the oxidation of 1 μmol of NADPH per min.

## RESULTS

**Expression of synthetic pathways in *E. coli* leads to isopropanol production.** We compared five different gene combinations for isopropanol production (Fig. 2). All synthetic pathways produced isopropanol from an initial glucose concentration of 111 mM (20 g/liter) under aerobic conditions. For all combinations, glucose was exhausted within 12 h after inoculation (9 h after induction with IPTG). As shown in Fig. 2, TA11 with pTA39/pTA36 produced the greatest amount of isopropanol. The amounts of ethanol produced by all strains were very small (less than 10 mM) compared with the isopropanol production.

Figure 3 shows the time course of fermentation using TA11(pTA39/pTA36). The isopropanol concentration decreased after the exhaustion of glucose (12 h). Addition of glucose (111 mM) to the culture at 24 h restored isopropanol production to the same rate as in the first production phase, indicating that the pathway activity was stable even after 14 h of starvation. When glucose was exhausted (30.5 h), the final concentration of isopropanol achieved was 81.6 mM. The acetone concentration continued to increase after exhaustion of the initial glucose and suddenly decreased with the addition of glucose (Fig. 3). No organic acids except fumaric acid (maximum concentration, 386 μM) accumulated significantly after induction by IPTG.

***C. beijerinckii* SADH performs better than *T. brockii* SADH.** We compared isopropanol production levels from the strains with pTA36 or pTA18. The amino acid sequence of SADH from *C. beijerinckii* has 76% identity and 86% similarity with that from *T. brockii* (18). However, TA11(pTA39/pTA18) produced lower concentrations of isopropanol and much higher concentrations of acetone than the strain containing *adh* from *C. beijerinckii*(pTA39/pTA36). To investigate further, the enzyme activities in crude extracts from cultures containing these two alcohol dehydrogenases (either pTA18 or pTA36) were measured. The activity of alcohol dehydrogenase from *C. beijerinckii*(pTA39/pTA36) (3.08 ± 0.36 units/mg of protein) was

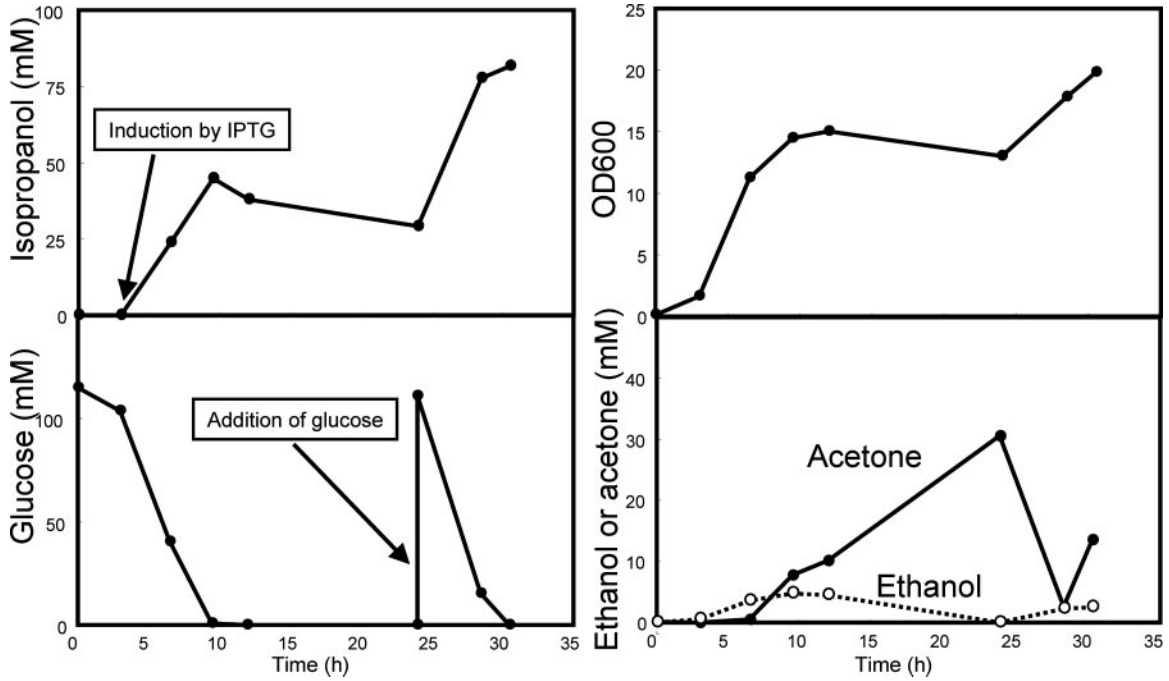



FIG. 3. Time course of isopropanol production by TA11(pTA39/pTA36).

indeed much higher than that from *T. brockii*(pTA39/pTA18) (0.24 ± 0.08 unit/mg of protein), consistent with the higher isopropanol production.

**Acetone production.** When the *adh*-containing plasmid was omitted from the strain, the host produced acetone by TA11 containing only pTA39 under the same conditions as those for the isopropanol production experiment. The strain continued to produce acetone until the glucose was exhausted (12 h). At 24 h, glucose was added to 111 mM, and the strain continued to produce acetone at almost the same production rate (data not shown). The cell growth and ethanol concentration displayed a tendency similar to that observed during the isopropanol production experiment. When glucose was exhausted (30.5 h), the final concentration of acetone was measured at 148.3 mM. As with the isopropanol production, no organic acids except fumaric acid (maximum concentration, 292 μM) accumulated significantly after the induction of IPTG.

## DISCUSSION

In this study, we achieved the first isopropanol production in *E. coli*. In shake flask cultures, strain TA11 containing pTA39/pTA36 produced 81.6 mM isopropanol at 30.5 h with a maximum productivity of 6.9 mM/h (0.41 g/liter/h) between 3 and 9.5 h. The engineered *E. coli* surpassed the best reported strain of *C. beijerinckii*, NRRL B593, which produces isopropanol at ~30 mM with a maximum productivity of ~3 mM/h (~0.18 g/liter/h) (4). The isopropanol yield at 9.5 h after inoculation was 43.5% (mol isopropanol/mol glucose). The yield was calculated from the isopropanol produced (44.8 mM) and the glucose consumed (103.0 mM) between 3 and 9.5 h: molar yield = 44.8/103.0 = 0.435. This yield is very encouraging, since the maximum theoretical yield from glucose using our production pathway is 1 mol isopropanol/mol glucose. The theoretical yield of isopropanol production was calculated based on the pathway shown in Fig. 1. One mole of glucose is converted to 2 mol acetyl-CoA and 2 mol of $CO_2$. The two acetyl-CoAs are then condensed to form 1 mole of isopropanol, losing one additional mole of $CO_2$.

Our results showed that *E. coli* containing *atoAD* produced isopropanol at a higher concentration than the strain containing *C. beijerinckii ctfAB*. As Wiesenborn et al. (24) pointed out, the $K_m$ for acetate for CtfAB (1,200 mM) is much higher than that for AtoAD (53.1 mM) (1, 20). This difference in acetate affinity likely explains why AtoAD is a better enzyme for isopropanol production. Isopropanol production showed that the conversion rate of SADH from *C. beijerinckii* from acetone to isopropanol is much higher than that of SADH from *T. brockii*. Indeed, the alcohol dehydrogenase assay using crude extract showed that SADH from *C. beijerinckii* has a higher activity for isopropanol formation from acetone than that from *T. brockii*, consistent with the results of Peretz et al. (18). Although a difference in expression levels cannot be ruled out, the SADH from *C. beijerinckii* is a better choice for isopropanol production under our conditions. We did not investigate the exact effect of codon optimization in *adh* genes. However, it is generally known that the GC content of the gene affects the expression efficiency. The GC contents of SADH in *C. beijerinckii* and in *T. brockii* are 38 and 44%, respectively. These values are different from the average GC content of *E. coli* K-12 MG1655.

Bermejo et al. (1) demonstrated batch and glucose-fed batch cultures using a shake flask for acetone production in *E. coli*, utilizing a recombinant acetone pathway from *C. acetobutylicum* (*thl ctfAB adc*). This strain produced about 40 mM acetone at 24 h after inoculation in batch culture and 93 mM

acetone in a glucose-fed batch culture. We also used the same medium, the same glucose concentration, and a modified version of *E. coli* strain B. Nevertheless, our batch production using the same gene construction (pTA41) achieved 60.3 mM of acetone at 12 h (data not shown). In shake flasks, strain TA11 containing pTA39 achieved a maximum concentration of 148.3 mM at about 30 h and a maximum productivity of 12.1 mM/h (0.70 g/liter/h) at 3 to 9.5 h. The acetone titer also exceeded that produced by wild-type *C. acetobutylicum* (90 mM) (1). Without further optimization of the strain and the fermentation conditions, the acetone yield at 12 h after inoculation was already 73.5% (mol/mol) of the theoretical maximum. This high yield indicates a great potential for using metabolically engineered *E. coli* in the industrial production of isopropanol.

## ACKNOWLEDGMENT

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