Mol Biotechnol
DOI 10.1007/s12033-016-9929-y

ORIGINAL PAPER

3-Methyl-1-butanol Biosynthesis in an Engineered *Corynebacterium glutamicum*

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Abstract Biofuel offers a promising solution to the adverse environmental problems and depletion in reserves of fossil fuels. Higher alcohols including 3-methyl-1-butanol were paid much more attention as fuel substitute in recent years, due to its similar properties to gasoline. In the present work, 3-methyl-1-butanol production in engineered *Corynebacterium glutamicum* was studied. α-Ketoisovalerate decarboxylase gene (*kivd*) from *Lactococcus lactis* combined with alcohol dehydrogenase gene (*adh2, adhA*, and *adh3*) from three organisms were overexpressed in *C. glutamicum.* Enzymatic assay and alcohol production results showed that *adh3* from *Zymomonas mobilis* was the optimum candidate for 3-methyl-1-butanol production in *C. glutamicum*. The recombinant with *kivd* and *adh3* could produce 0.182 g/L of 3-methyl-1-butanol and 0.144 g/L of isobutanol after 12 h of incubation. Further inactivation of the E1 subunit of pyruvate dehydrogenase complex gene (a*ceE*) and lactic dehydrogenase gene (*ldh*) in the above *C. glutamicum* strain would improve the 3-Methyl-1-butanol titer to 0.497 g/L after 12 h of incubation.

Keywords 3-Methyl-1-butanol · *Corynebacterium glutamicum* · α-Ketoisovalerate decarboxylase · Alcohol dehydrogenase · Gene knockout

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Introduction

The development of alternative green energy has gained increasing attention worldwide due to the exhaustion of petroleum resources, aggravating environmental pollution, and global warming [16, 24]. Recently, there has been growing research interest on the production of higher alcohol as fuel [23]. Many studies have shown that higher alcohols, e.g., 3-methyl-1-butanol, possess many advantages, such as, higher energy density, lower vapor pressure, and hygroscopicity, which are closer to the properties of gasoline than ethanol [14]. As a potential substitution for petroleum fuels, the use of 3-Methyl-1-butanol can reduce the global reliance on fossil fuels and mitigate possible fossil fuel energy shortages as well as environmental protection.

Previously, there have been limited numbers of reports with higher alcohols directly synthetized from glucose through the metabolism of microorganisms. In 2008, Liao reported firstly the non-fermentative pathways for biosynthesis of branched-chain higher alcohols as biofuels [2]. The common precursor α-keto acid for the amino acid biosynthesis can be converted into higher alcohols by α-keto acid decarboxylase (KDC) and alcohol dehydrogenase (ADH) through non-fermentative pathway. Over these years, researchers took advantage of this pathway to express relevant genes and eliminate competitive ways to synthesize higher alcohols, such as 1-propanol [3], 1-butanol [1, 29], and 3-methyl-1-butanol [11, 12, 27]. However, there are still many problems existing for higher alcohol production, including the toxicity of high concentrations of higher alcohols to cells, the overexpression of key genes, carbon flux directing to higher alcohols, and so on.

C. glutamicum is a gram-positive, facultatively anaerobic microorganism and has thick cell wall [21], which can

Published online: 09 March 2016

tolerate higher concentration of higher alcohols than *Escherichia coli* [6, 29]. *C. glutamicum* also acts as a natural workhorse for production of numbers of amino acids [30]. In the present work, *C. glutamicum* ATCC13032 was selected as host to eliminate the toxicity inhibition of higher alcohols. Ribosome binding site (rbs) [19] was added to optimize *kivd* expression levels, and *adh2* from *Saccharomyces cerevisiae* S288C, *adh3* from *Zymomonas mobilis* UQM410, and *adhA* from *Lactococcus lactis* IFPL730 were cloned for 3-methyl-1-butanol production in *C. glutamicum.* The activities of three *adhs* were also studied. Simultaneously, we also made the inactivation of pyruvate dehydrogenase gene *aceE* and lactic dehydrogenase *ldh* in *C. glutamicum* ATCC13032 to direct carbon flux for 3-methyl-1-butanol production. The route for 3-methyl-1-butanol biosynthesis is shown in Fig. 1.

Materials and Methods

Oligonucleotides, Plasmids, and Bacteria Strains

Kivd gene was obtained by the polymerase chain reaction (PCR) from genomic DNA of *L. lactis* IFPL730. *Adh2* gene was from *S. cerevisiae* S288C, *adhA* gene was from *L. lactis* IFPL730, and *adh3* gene was from *Z. mobilis* UQM410. *AceE* and *ldh* were inactivated in *C. glutamicum* ATCC13032 by crossover PCR. The characteristics of oligonucleotides were summarized in Table 1.

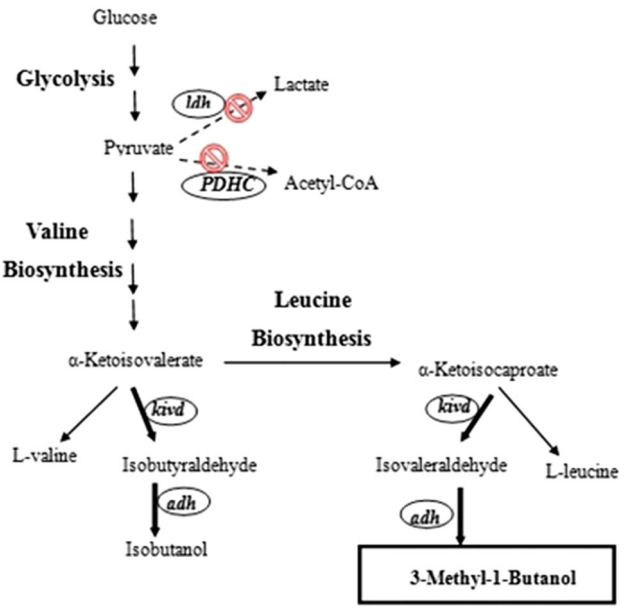

Fig. 1 Metabolic pathway of 3-Methyl-1-butanol biosynthesis. *Kivd* α-Ketoisovalerate decarboxylase, *adh* alcohol dehydrogenase, *ldh* lactic dehydrogenase, *PDHC* Pyruvate dehydrogenase complex. The light line stands for existing metabolic pathways in *C. glutamicum* and the rude line is on behalf of competing metabolic pathways, the imaginary line is knockout pathways

Plasmid pEASY-T1 was used for cloning *kivd(rbs)*, *adh*, Δ*aceE*, and Δ*ldh* genes. Plasmid pEA-kivd, pEA-adh, pEA-ΔaceE, and pEA-Δldh were constructed by A-T lining from pEASY-T1 and *kivd(rbs)*, *adh*, Δ*aceE,* and Δ*ldh*, respectively. Plasmid pEC-XK99E was used for expressing *kivd* and *adh* genes. Plasmid pEC-kivd-rbs was constructed by ligating big fragments of pEC-XK99E after digestion with *Sal*I and *Bam*HI and small fragments of pEA-kivd after digestion with the same restriction enzyme. Plasmid pEC-kivd-rbs-adh and pEC-adh were constructed by the same method. The suicide plasmid pK18mobsacB was suitable for gene knockout in *C. glutamicum*. The resulting fusion product ΔaceE and Δldh were ligated into SalI-BamHI digested plasmid pK18mobsacB and transformed into *E. coli* for plasmid propagation. The recombinant plasmid was isolated from *E. coli* and transferred into *C. glutamicum* by electrotransformation method [31]. Colonies were firstly inoculated into LBHIS medium (0.5 % typtone, 0.25 % yeast extract, 0.5 % NaCl, 1.85 % brain heart infusion, 9.1 % sorbitol) with 50 μg/mL of kanamycin, and secondly incubated into LBHIS medium with 10 % sucrose. In addition, the inactivation of *aceE* and *ldh* were further confirmed by assaying the molecular lengths of these two gene by PCR using knocked *C. glutamicum* (data not shown). By application of the homologous recombination (double crossover) [25] and counter-selection, the intact chromosomal *aceE* and *ldh* genes in *C. glutamicum* were replaced by ΔaceE and Δldh. The traits of plasmids were summarized in Table 2.

E.coli Trans1-T1 was used for propagating plasmid. The constructed plasmids were transformed into *E.coli* by heat shock. *C. glutamicum* ATCC13032 was used as host strain for production of isovaleraldehyde and 3-methy-1-butanol. The resulting plasmids were transformed into *C. glutamicum* by electrotransformation. The features of bacteria strains were summarized in Table 3.

Reagents and Growth Conditions

Oligonucleotides were ordered from Invitrogen (Shanghai, China). All restriction enzymes and rapid DNA ligation kit were purchased from Takara(Dalian, China). Trans Start Taq DNA polymerase was purchased from TRANS (Beijing, China).

E. coli was cultured in LB medium (5 g/L yeast extract, 10 g/L tryptone, and 10 g/L NaCl), *L. lactis* was cultured in LBG medium (5 g/L yeast extract, 10 g/L tryptone, and 10 g/L NaCl, 5 g/L glucose), *S. cerevisiae* was cultured in YPD medium (10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose), *Z. mobilis* was cultured in LBG medium, and their cells routinely grew at 37 °C. *C. glutamicum* was

Table 1 Primers used in this work

Cloning gene	Primer name	Sequence (5′ → 3′)	Restriction enzyme
kivd	K1	CGCGGATCCATGTATACAGTAGGAG	*BamH*I
	K3	GTCGACTTATGATTTATTTTGTTCAGCAAAT	*Sal*I
kivd-rbs	K2	CGCGGATCC AAAGGAGGACAACCATGTATACAGTAGGAG	*BamH*I
	K3	GTCGACTTATGATTTATTTTGTTCAGCAAAT	*Sal*I
adh2	A1	GCGGTACCATGTCTATTCCAGAAACTCAAAA	*Kpn*I
	A2	GGATCCTTATTTAGAAGTGTCAACAACGTAT	*BamH*I
adhA	A3	GCGGTACCATGAAAGCAGCAGTAGTAAGAC	*Kpn*I
	A4	TCCGGATCC TTATTTAGTAAAATCAATGACCA	*BamH*I
adh3	A5	GGGGTACC ATGGCTTCCTCAACTTTTTAT	*Kpn*I
	A6	CGGGATCCTTAGAAAGCGCTCAGGAAG	*BamH*I
Δ*aceE*	f1	ACGCGTCGACATGGCCGATCAAGCAAAACTTGG	sal1
	f2	GGATAGGTGATTGGAAGTTGGCGTGCGTACATACCTG	
	f3	CCAACTTCCAATCACCTATCCCCATGAGGCAAACATCTTG	
	f4	CGCGGATCCTTATTCCTCAGGAGCGTTTGGATCT	*BamH*1
Δ*ldh*	f5	ACGCGTCGACATGAAAGAAACCGTCGGCAAT	sal1
	f6	GGATAGGTGATTGGAAGTTGGCGGCACAAATGACAACC	
	f7	CCAACTTCCAATCACCTATCCCCCAGAGCTTGAGGGC	
	f8	CGCGGATCCTCAGAAGAACTGCTTCTGAA	*BamH*1

Note underline ∼ base is RBS

Table 2 Plasmids used in this work

Plasmids	Relative feature	Source
pEASY-T1	Kan + Amp + LacZ pUCori	TRANS
pEA-kivd	Kan + Amp + LacZ pUCori kivd	This work
pEA-kivd-rbs	Kan + Amp + LacZ pUCori kivd-rbs	This work
pEA-adh2	Kan + Amp + LacZ pUCori adh2	This work
pEA-adhA	Kan + Amp + LacZ pUCori adhA	This work
pEA-adh3	Kan + Amp + LacZ pUCori adh3	This work
pEA-ΔaceE	Kan + Amp + LacZ pUCori ΔaceE	This work
pEA-Δldh	Kan + Amp + LacZ pUCori Δldh	This work
pEC-XK99E	per repA aph(3′)-IIa oriV lacIq Ptrc	Kirchner and Tauch, [18]
pK18mobsacB	Kan + *oriT* oriV sacB	Schäfer et al. [25]
pEC-kivd	per repA aph(3′)-IIa oriV lacIq Ptrc kivd	This work
pEC-kivd-rbs	per repA aph(3′)-IIa oriV lacIq Ptrc kivd-rbs	This work
pEC-adh2	per repA aph(3′)-IIa oriV lacIq Ptrc adh2	This work
pEC-adhA	per repA aph(3′)-IIa oriV lacIq Ptrc adhA	This work
pEC-adh3	per repA aph(3′)-IIa oriV lacIq Ptrc adh3	This work
pEC-kivd-rbs-adh2	per repA aph(3′)-IIa oriV lacIq Ptrc kivd-rbs adh2	This work
pEC-kivd-rbs-adhA	per repA aph(3′)-IIa oriV lacIq Ptrc kivd-rbs adhA	This work
pEC-kivd-rbs-adh3	per repA aph(3′)-IIa oriV lacIq Ptrc kivd-rbs adh3	This work
pK-ΔaceE	Kan + *oriT* oriV sacBΔaceE	This work
pK-Δldh	Kan + *oriT* oriV sacBΔldh	This work

cultured in LBG medium with 30 °C, 50 μg/mL kanamycin and/or 1 mmol/L isopropyl-β-D-thiogalactoside (IPTG) were also used for screening and inducing. Recombinants C10 and C11 were incubated on LBG medium.

Detection of Metabolites

C. glutamicum recombinants were streaked into LBG solid plate containing 50 μg/mL kanamycin. A single colony was

Table 3 Strains used in this work

Strains	Relevant characteristic	Source
E.coli		
Trans1-T1	*F^{-}φ80 (lac Z)ΔM15 ΔlacX74 hsdR17(r_k^-,m_k^+) ΔrecA1398 endA1 tonA*	TRANS
C. glutamicum		
ATCC13032	Wide type	American type culture collection
C1	ATCC13032/pEC-XK99E	This work
C2	ATCC13032/pEC-kivd	This work
C3	ATCC13032/pEC-kivd-rbs	This work
C4	ATCC13032/pEC-kivd-rbs-adh2	This work
C5	ATCC13032/pEC-kivd-rbs-adhA	This work
C6	ATCC13032/pEC-**k**ivd-rbs-adh3	This work
C7	ATCC13032/pEC-adh2	This work
C8	ATCC13032/pEC-adhA	This work
C9	ATCC13032/pEC-adh3	This work
C10	ATCC13032ΔaceE	This work
C11	ATCC13032ΔaceEΔldh	This work
C12	C10/pEC-**k**ivd-rbs-adh3	This work
C13	C11/pEC-**k**ivd-rbs-adh3	This work
L.lactis	Wide type	China Center of Industrial Culture Collection
S.cerevisiae	Wide type	China Center of Industrial Culture Collection
Z.mobilis	Wide type	China Center of Industrial Culture Collection

pre-cultured in test tubes containing 3 mL of LBG medium under 30 °C overnight in a rotary shaker (200 rpm). Overnight culture was diluted with ratio of 1:100, into 10 mL of fresh LBG medium in a 50-mL plastic test tube, and three parallel experiments were performed. Cells grew at 30 °C for 3–4 h (OD_{600} up to 0.6–0.8), followed by adding 1 mM isopropyl-β-D-thiogalactoside (IPTG). The samples from supernatant were collected after 12 h for metabolites detection. The produced alcohol compounds were quantified by gas chromatography (GC-2014, Shimadzu) with flame ionization detector. The GC procedure was as follows: oven temperature was initially held at 60 °C for 1 min, and raised with a gradient of 10 °C/min to 260 °C, then held for 2 min. The detector was maintained at 250 °C. A 1:50 split ratio was performed. 1 μL sample was injected after dealing with 0.22-μm filter membrane. The hydrogen pressure was 0.4 MPa, air pressure was 0.4 MPa, and argon pressure was 0.6 MPa, respectively.

Protein Quantification and *Adh* Enzyme Activity Assay

adh2, *adhA*, and *adh3* gene were overexpressed in *C. glutamicum* with plasmid pEC-adh. The cultured cells were collected by centrifugation and washed with 500 μL double-distilled water and suspended with 600 μL of 50 mM phosphate buffer (pH 6.5). The resuspended cells were broken with ultrasonic waves with 5 s pulses until the suspended liquid became clear. The samples were then centrifuged for 15 min (12,000×*g*, 4 °C). Supernatant protein was quantified with the method of Bradford [8] and SDS-PAGE. Alcohol dehydrogenase (Adh) activity was assayed as described [4]. Enzymatic reactions (0.25 mL) were performed with 50 mmol/L 3-(N-morphplino) propanesulfonic acid buffer, pH 7.0, 0.25 mmol/L NADH, 30 mmol/L substrate (acetaldehyde, isobutyraldehyde, and isovaleraldehyde), and 0.25 mL *adh* protein crude enzyme fluid. The reaction solution was kept at 37 °C for 20 min, then measured the absorbance at 260 nm using a spectrophotometer. One unit of enzyme activity was defined as the amount (mL) of crude protein which got the increase of 0.01 unit at OD_{260} in reaction solution, at 37 °C.

Results and Discussion

Effects of *kivd* on Isovaleraldehyde Production

The production of higher alcohol from α-keto acid needs the synergistic work of α- ketoisovalerate decarboxylase and alcohol dehydrogenase. In previous reports, overexpression of *kivd* gene under plasmid pZA31-luc [20] and pSA55 [3] could produce isobutyraldehyde rather than isovaleraldehyde. *Kivd* from gram-positive microogranism *L. lactis* was reported for the decarboxylation of α-keto acids derived from amino acid transamination into aldehydes [9, 13, 15, 17]. In our previous studies, it has been proved that *kivd* can convert α-ketoisocaproic acid into

isovaleraldehyde in *E. coli* [10]. In present study, *kivd* gene was overexpressed with plasmid pEC-XK99E in *C. glutamicum* (C2), and trace of isovaleraldehyde was detected in the fermentation broth.

It had been previously observed [28] that a short terminal section of the 3′-end of small ribosomal RNA of *E. coli*, known as ribosomal binding site (rbs), was able to interact with mRNA and involve in the termination and initiation of protein biosynthesis. To increase the expression of *kivd* in *C. glutamicum* (C3), a ribosomal binding site was added to the 5′-end of *kivd* gene initiation codon. With the addition of rbs (C3), isovaleraldehyde concentration could attain to 0.063 g/L, and the concentration of isobutanol or 3-methyl-1-butanol was too low to be detected. In our work, the decarboxylation reaction of *kivd* with rbs was more efficient than *kivd* without rbs, which was consistent with the report about rbs increasing the expression of target genes [22].

Production of 3-methly-1-butanol

As for branched-chain higher alcohols biosynthesis through non-fermentative pathways, alcohol dehydrogenase (*adh*) is responsible for the reduction of isovaleraldehyde to 3-methyl-1-butanol. To obtain higher yield of alcohols in *C. glutamicum*, three alcohol dehydrogenase genes, *adh2* from *S. cerevisiae*., *adhA* from *L. lactis*, and *adh3* from *Z. mobilis* were separately ligated with *kivd* gene in plasmid pEC-kivd-rbs, resulting three recombinants named as strain C4, C5, and C6, respectively. The alcohol production of these strain varied from each other. Strain C4 harboring *adh2* did not produce any 3-methyl-1-butanol, and the production of isovaleraldehyde declined to 0.028 g/L, strain C5 with *adhA* produced 0.022 g/L of isovaleraldehyde and 0.81 g/L of isobutanol, and the yield of 3-methyl-1-butanol was too low to be detected, recombinant C6 containing *adh3* produced 0.144 g/L of isobutanol and 0.182 g/L of 3-methyl-1-butanol. These results suggest that *adh3* has better preference on the reduction of isovaleraldehyde, which is the first report that *adh3* was more suitable for the hydrogenation reaction of isovaleraldehyde.

Overexpression of the pathway-specific genes is the most readily accessible strategies in metabolic engineering. Previous reports [4, 29] have utilized *kivd* and *adh2* or *adhA* genes for the production of isobutanol. In the present study, *kivd* and *adh3* genes were used to increase the production of 3-methyl-1-butanol in *C. glutamicum*, the results showed that recombinant C6 strain can serve as an ideal candidate for 3-methyl-1-butanol production.

Properties analysis of alcohol dehydrogenase

To analyze the properties of *adh2*, *adhA*, and *adh3*, three adh genes were cloned into plasmid pEC-XK99E and expressed in *C. glutamicum,* generating recombinants C7, C8, and C9, respectively. The protein content of crude enzyme of each strain is shown in Table 4. Compared to C1 protein content of 0.209 mg/mL, the protein contents of recombinants C7, C8, and C9 increased to 0.336, 0.295, and 0.333 mg/mL, respectively. The protein expression was assayed by SDS-PAGE, and results showed that three *adh* genes were successfully expressed in *C. glutamicum* (Fig. 2).

Table 4 The protein content of crude extract

Strain names	Overexpression	Protein content (mg/mL)
C1	–	0.209
C7	adh2	0.333
C8	adhA	0.336
C9	adh3	0.295

To verify the substrate specificity of *adh*, acetaldehyde, isobutyraldehyde, and isovaleraldehyde were used as the substrates to assay enzyme activity (Fig. 3). Crude extract of C1 could slightly catalyze the dehydrogenation reaction, which manifested that wild *C. glutamicum* strain contains alcohol dehydrogenase. Strain C7 containing a*dh2* showed the highest dehydrogenase activity on acetaldehyde, its dehydrogenase performance on isobutyraldehyde or isovaleraldehyde is much lower than that of *adhA* and *adh3*. Strain C8 containing *adhA* showed great preference for isobutyraldehyde conversion, its dehydrogenase ability on isovaleraldehyde is almost similar to parent *C. glutamicum* strain C1. Strain C9 containing *adh3* presented much better reduction ability on all the three aldehydes than original strain, especially on isovaleraldehyde. Meanwhile, the results of enzyme assays are in agreement with 3-methyl-1-butanol and isobutanol data from strains C4, C5, and C6, which also indicated strain C6 was the ideal candidate for 3-methyl-1-butanol production.

Deletion of *aceE* and *ldh* to Increase 3-methyl-1-butanol Production

Pyruvate, the precursor of amino acid biosynthesis, is also the common substrate of other metabolic pathway. Pyruvate dehydrogenase complex (*PDHC*) can convert pyruvate into acetyl-CoA, which then be converted to acetic acid. Pyruvate is also the intermediate of lactic acid. The competition pathway on pyruvate metabolism will weaken the carbon flux to α-keto acid, and lower the yield of higher alcohol. Previous reports had showed the improvement of higher alcohol production by gene deletion of *aceE* and *ldh* gene, which is responsible for pyruvate metabolism to acetic acid and lactic acid, respectively [5, 26]. In present study, *C. glutamicum* recombinant strain C10 and C11

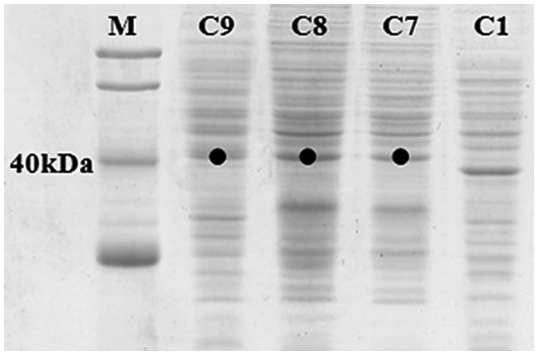

Fig. 2 SDS-PAGE results of protein for recombinants. M is protein marker, consisting of 80, 60, 40, and 30 kDa proteins. *Filled circle* is the position of alcohol dehydrogenase. The C1 strain is *C. glutamicum* ATCC13032 with plasmid pEC-XK99E; *Adh2* gene was expressed in C7 strain; *adhA* gene was expressed in C8 strain; *adh3* gene was expressed in C9 strain

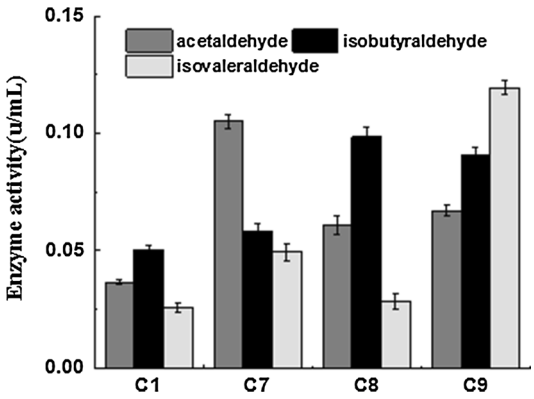

Fig. 3 Effects of different substrates on dehydrogenase activity in crude extract. The enzymatic reactions system consists of 3-(N-morphplino) propanesulfonic acid buffer, NADH, substrate (aldehyde, isobutyraldehyde, and isovaleraldehyde), and *adh* protein crude enzyme fluid (C1, C7, C8, and C9), which keeps at 37 °C for 20 min

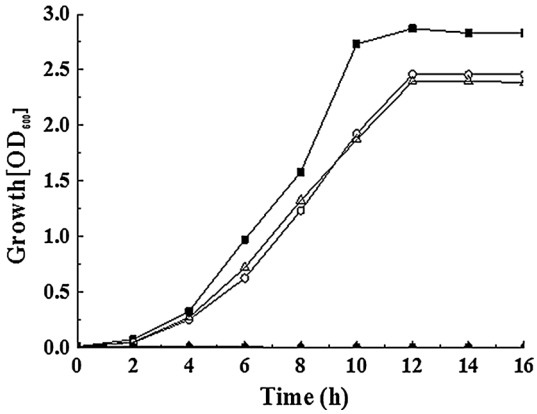

Fig. 4 Strain growth curve of recombinants cultivated with LBG, 30 °C, 150 rpm. *Filled square* C1, *open circle* C10, *open triangle* C11

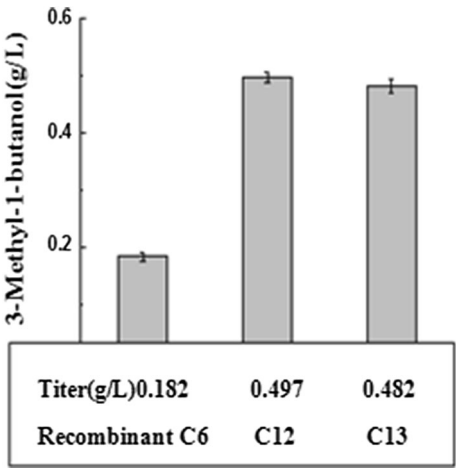

Fig. 5 Yield of 3-methyl-1-butanol in *C. glutamicum* recombinants. The recombinants were incubated in LBG medium containing 5 g/L yeast extract, 10 g/L tryptone, 10 g/L NaCl, and 5 g/L glucose, at 30 °C, induced with 0.1 mM IPTG for 12 h

were constructed by inactivation of *aceE* alone, and inactivation of *aceE* and *ldh* simultaneously, respectively. *C. glutamicum* recombinants C10, C11, and wide type (C1) were streaked into LBG. Bacterial strain C10 and C11 grew well on LBG (Fig. 4), which was different from the former report that *aceE*-deficient *C. glutamicum* strain required either acetate or ethanol as additional carbon source for growth [7]. We speculate that LBG medium with glucose containing growth factor could satisfy the growth of mutation strains.

In strain C10 and C11 fermentation broth, little amount of alcohol was detected. In our continuous studies, plasmid pEC-kivd-rbs-adh3 was transformed into C10 and C11 to form C12 and C13. After 12 h of incubation induced with IPTG, recombinant C12 could produce 0.497 g/L of 3-methyl-1-butanol, and C13 (Fig. 5) also could produce 0.482 g/L of 3-methyl-1-butanol.

Our results showed the alcohol production with *aceE* gene deletion in *C. glutamicum* increased 2.7-fold compared to the original strain, which agreed with the results of previous reports [6]. However, further inactivation of *ldh* gene did not improve 3-methyl-1-butanol yield, 0.287 g/L of isobutanol was detected with strain C12 and 0.336 g/L of isobutanol was detected with strain C13, which suggested that *ldh* gene deletion was a benefit for the carbon flux toward α-keto acid biosynthesis. The biosynthesis of 3-methyl-1-butanol needs another series of enzymatic reactions based on α-ketoisovaleric acid metabolism, which hindered the form speed of 3-methyl-1-butanol. That is the main reason why the unchanged amount of 3-methyl-1-butanol was formed by C13 with *ldh* inactivation based on C12.

Conclusion

A series of recombinants with *C. glutamicum* were successfully constructed via overexpression of *kivd* and *adh3* genes and deletion of *aceE* and *ldh* for over-production of 3-methyl-1-butanol. The recombinant C12 inoculating in LBG medium could produce 0.497 g/L of 3-methyl-1-butanol after 12 h of incubation. To our knowledge, this is also the maximum reported case for 3-methyl-1-butanol production in *C. glutamicum*.

Acknowledgments This research was financially supported by the National Natural Science Foundation of China (No. 2117623 and No.21211140237), National High Technology Research and Development Program of China (863 Program) (2013AA065803), Guangdong science and technology research program (2013B010403021).

Compliance with Ethical Standards

Conflict of interest The author(s) Shiyuan Xiao, Jingliang Xu, Xiaoyan Chen, Xiekun Li, Yu Zhang, Zhenhong Yuan declare that they have no competing interests.

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