The draft genome sequence of *Clostridium* sp. strain CT7, an isolate capable of producing butanol but not acetone and 1,3-propanediol from crude glycerol

Jiasheng Lu¹ · Tianpeng Chen¹ · Yujia Jiang¹ · Wenming Zhang^{1,2} · Weiliang Dong^{1,2} · Jie Zhou¹ · Jiangfeng Ma^{1,2} · Yan Fang^{1,2} · Min Jiang^{1,2} · Fengxue Xin^{1,2}

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Abstract

A solventogenic *Clostridium* sp. strain CT7 which could utilize glycerol directly to produce high yields of butanol was isolated. In the presence of crude glycerol, strain CT7 synthesized butanol through a unique butanol–ethanol (BE) fermentation pathway in which acetone and 1,3-propanediol (1,3-PDO) were not produced. The genome of strain CT7 which has a G + C content of 30.3% was estimated to be 5.99 Mb and contained 4319 putative Open Reading Frames (ORF). The putative annotated genes, which play major roles in BE production from crude glycerol, included glycerol dehydrogenase gene (*gdh*), acetoacetyl-CoA transferase gene (*ctfA/B*), and bifunctional alcohol and aldehyde dehydrogenase gene (*adhE*). In addition, non-typical BE production is not coupled to 1,3-propanediol formation, which may due to the defect of 1, 3-PDO dehydrogenase gene (*dhaT*).

Keywords Glycerol · Butanol · *Clostridium* · By-products · *AdhE* · Draft genome sequencing

Due to the concerns regarding depleting stock and higher prices of fossil oil, production of biofuel has been generating great interest and promise (Lee et al. 2010). Butanol shows more promising properties to ethanol as it has higher energy density and octane number, lower hydrophilicity, and

volatilization (Shanmugam et al. 2018a). Biobutanol is usually produced through the traditional acetone–butanol–ethanol (ABE) fermentation process with a mass ratio of 3:6:1 typically by solventogenic *Clostridium* species (Wang et al. 2013; Sun et al. 2018), such as *C. acetobutylicum* and *C. beijerinckii* (Shanmugam et al. 2018b; Jiang et al. 2018). As the current ABE fermentation process utilizes molasses and starch as substrates, but the high cost of these raw materials hinders commercial production of butanol (Jiménez-Bonilla and Wang 2017; Sarchami and Rehmann 2014), and consequently, there is urgency in finding alternate inexpensive substrates, such as organic wastes (Xin et al. 2017).

Crude glycerol is a major by-product of the biodiesel industry, and is considered as an alternative substrate for biofuel and biochemical production. The content of glycerol in crude glycerol is 82–90%. With the expansion in the scale of biodiesel production, an excess glycerol is being produced annually (Xin et al. 2016). Thus, the adoption of waste glycerol for butanol production can not only reduce butanol production costs, but also effectively solve the problem of glycerol overproduction. In general, glycerol is first converted to dihydroxyacetone phosphate, and then driven into the glycolysis pathway under the catalysis of glycerol kinase and glycerol phosphate dehydrogenase (Huang et al.

Jiasheng Lu and Tianpeng Chen are contributed equally to this work.

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✉ Min Jiang
bioengine@njtech.edu.cn

✉ Fengxue Xin
xinfengxue@njtech.edu.cn

¹ State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Puzhu South Road 30#, Nanjing 211800, People's Republic of China

² Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing Tech University, Nanjing 211800, People's Republic of China

2015). Unfortunately, only a few solventogenic *Clostridium* sp. can directly utilize glycerol for butanol production. Currently, *C. pasteurianum* is the only reported solventogenic *Clostridium* sp. that possess glycerol metabolism pathway. Nevertheless, by-products, such as 1,3-Propanediol (1,3-PDO) in the fermentation process, also greatly reduce the butanol yield and increase cost as butanol has to be separated from 1,3-PDO (Taconi et al. 2009).

A new *Clostridium* sp. strain CT7 isolated from a sample of decomposite soil from Qixiashan Nature Park, Nanjing, China, in our lab was able to produce butanol from glycerol. After medium and process optimization, strain CT7 was found to produce 16.6 g/L of butanol from 60 g/L of glycerol and 11.8 g/L of butanol from 60 g/L crude glycerol. Interestingly, acetone and 1,3-PDO were not detected from glycerol fermentation during butanol production, which was suggested that strain CT7 could synthesize butanol through a unique butanol-ethanol (BE) fermentation pathway (Fig. 1). More importantly, only small amount of ethanol (0.8 g/L) was produced, contributing to the mass ratio of butanol:ethanol up to 20:1, which is much higher than the typical 6:1.

To better elaborate the unique metabolic properties of strain CT7, the whole genome of strain CT7 was sequenced and is presented here. Mineral salt medium amended with glycerol as sole carbon source was used to cultivate strain CT7 for 3 days and its genomic DNA was extracted using G⁺ bacteria genomic DNA kit (ZOMANBIO, China) (Jiang et al. 2017). The DNA libraries were constructed using NEBNext® UltraTM DNA Library Prep Kit for Illumina (NEB, USA) and sequenced using Illumina HiSeq 2500

system sequencing technology (Jiang et al. 2017). Raw reads were trimmed and filtered using Trimmomatic v0.32, and the quality score Q30 is 93.20%. The filtered reads were then used to assemble the genome using Velvet v1.2.03 (Zerbino and Birney 2008). The Open Reading Frames (ORF) of the assembled genome were predicted using Glimmer3 v3.02 (Delcher et al. 2007).

Genome sequence blast showed that the 16S rDNA sequence of strain CT7 shares 99% similarity with the 16S ribosomal RNA sequence of *C. pasteurianum* GL11 in NCBI website (Table 1). Seventy-seven large contigs ranging from 4226 to 4,485,768 bases were generated by sequencing the genome. The sequence results clearly showed that no plasmids were detected and only one chromosomal DNA was contained in strain CT7. Strain CT7 possesses a smaller genome size than that from *C. beijerinckii* NRRL B-598 (6.0 Mb vs 6.2 Mb) (NCBI Accession Number: CP011966.1) (Table 2), a widely studied stain with the complete genomic sequences available (Sedlar et al. 2015). All contigs of CT7 were predicted to possess 4319 coding sequences (CDSs) with 30.3% G + C content, smaller than that from *C. beijerinckii* NRRL B-598, with 21 rRNA and 76 tRNA sequences.

Studies have shown that the best butanol producer from glycerol is *C. pasteurianum*, a Gram-positive, anaerobic, and non-pathogenic bacterium (Sarchami et al. 2016). The most crucial enzyme in glycerol utilization pathway is glycerol dehydrogenase (*gdh*), capable of catalyzing glycerol to pyruvate. In *Clostridium* sp. CT7, one *gdh* gene was found from its genomes according to the sequence data. Results revealed that *gdh* gene contains 1062 bp, which shows 79% identity with 100% coverage to that of *C. acetobutylicum* ATCC 824

Fig. 1 Proposed metabolic pathway for BE production within *Clostridium* sp. CT7. Dotted arrows indicate the deletion of the pathway

Table 1 Top matches of the 16S rRNA gene sequence of strain CT7 against known bacterial sequences from the Genbank database (BLAST, NCBI)

Microorganisms	Identity (%)
<i>C. pasteurianum</i> strain GL11 16S ribosomal RNA gene, partial sequence	99
<i>Clostridium</i> sp. strain WANY51 16S ribosomal RNA gene, partial sequence	99
<i>C. beijerinckii</i> NCIMB 8052, complete genome	99
<i>C. beijerinckii</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 7829	98
<i>Clostridium</i> sp. strain WANY51 16S ribosomal RNA gene, partial sequence	98
<i>C. roseum</i> gene for 16S rRNA, partial sequence, strain: N36	98

Table 2 Genome features comparison between *Clostridium* sp. strain CT7 and *C. beijerinckii* NRRL B-598 strain

Feature	<i>Clostridium</i> sp. strain CT7	<i>C. beijerinckii</i> NRRL B-598
Genome size (bp)	5,993,599	6,186,879
GC content (%)	30.3	29.8
Protein-coding genes (CDS)	4319	5002
rRNAs	21	49
tRNAs	76	94

(NCBI sequence accession: AE001437.1) through genome sequence blast in the NCBI website using non-redundant protein sequences database (Nr). It was suggested that this gene in strain CT7 may play the key role in glycerol utilization.

In the typical ABE fermentation pathway, acetoacetyl-CoA is catalyzed by acetoacetyl-CoA transferase (*ctfA/B*) and acetoacetate decarboxylase (*adc*) to form acetone (Fig. 2a) (Jiang et al. 2009). However, the phenomenon of acetone-uncoupled in strain CT7 indicated that these genes may be blocked or even deficient. Indeed, genomic blast results showed that one *adc* was found; however, no *ctfA/B* genes were detected, suggesting that although *adc* is present, the defect of key *ctfA/B* genes is the cause of the elimination of acetone (Table 3). Similar results were also found in the other strains. For example, *C. pasteurianum* DSM 525, the most extensively used strain for butanol production from glycerol, also showed no acetone formation because of the

deficiency of key *ctfA/B* gene (Table 3). In addition, it was demonstrated that *ctfA/B* plays an important role in the acid re-assimilation (Yu et al. 2015). Millat et al. found that *C. acetobutylicum* possessed a CoA-transferase-independent butyrate uptake mechanism which was activated by decreasing pH levels (Millat et al. 2014). Hence, it was speculated that butyrate could still be assimilated by the other means despite the defect of *ctfA/B* genes. 1,3-PDO dehydrogenase (*dhaT*) has been proved to be the key enzyme to catalyze 3-hydroxypropionaldehyde to 1,3-PDO, which is another by-product when glycerol was used as the substrate (Fig. 2b). In *C. pasteurianum* DSM 525, one *dhaT* was found, which is responsible for 1,3-PDO synthesis. Interestingly, no *dhaT* was found in the genome of strain CT7, which explains the elimination of 1,3-PDO formation. Taken altogether, the deficiency of key genes for the formation of acetone and 1,3-PDO in the genome of strain CT7 resulted in the non-typical BE fermentation pattern.

Table 3 Numbers of genes for acetone and 1,3-PDO metabolic pathways within different solventogenic *Clostridium* strains

Strains	Numbers of genes		
	<i>ctfA/B</i>	<i>adc</i>	<i>dhaT</i>
<i>C. beijerinckii</i> NCIMB 8052	4	1	0
<i>C. beijerinckii</i> NRRL B-598	3	1	0
<i>C. acetobutylicum</i> ATCC 824	2	1	0
<i>C. pasteurianum</i> DSM 525	0	1	1
<i>Clostridium</i> sp. strain CT7	0	1	0

Fig. 2 Arrangement of acetone, 1,3-PDO, ethanol, and butanol metabolic pathways within solventogenic *Clostridium* strains

Another noteworthy phenomenon is that the mass ratio of B:E (butanol:ethanol) by strain CT7 from glycerol is 20:1 (16.6 g/L:0.8 g/L), which is quite different from the typical ABE fermentation pattern of 6:1. It is well known that bifunctional alcohol and aldehyde dehydrogenase gene (*adhE*) are believed to be significant for the formation of alcohols (Bhandiwad et al. 2013). In general, AdhE is a bifunctional enzyme with two conserved domains, aldehyde dehydrogenase (*aldh*) domain and alcohol deoxidase (*adh*) domain (Lo et al. 2015). Therefore, AdhE can continuously catalyze the formation of acetaldehyde from acetyl-CoA, and convert acetaldehyde into ethanol, which is a key enzyme for ethanol metabolism (Fig. 2c). Similarly, AdhE can also convert butyl-CoA to butanol (Fig. 2d). In *Clostridium* sp. CT7, two *adhE* genes were found from its genomes according to the sequence report. One *adhE* gene contains 2577 bp, which shows 86% identity with the 99% coverage to *C. acetobutylicum* (NCBI sequence accession: WP_010890720.1) through genome sequence blast in the NCBI website using Nr data-based. Another *adhE* gene contains 2583 bp, which possesses 86% identity with 99% coverage to *C. acetobutylicum* (NCBI sequence accession: WP_010890846.1), indicating that these two hypothetical AdhEs are more likely the key enzymes for alcohol formation. The different affinity towards butyl-CoA compared with acetyl-CoA may contribute to the high butanol ratio.

Taken together, due to the efficient capability of glycerol utilization and the presence of hypothetical *adhE* genes, *Clostridium* sp. CT7 could produce high-yield butanol from glycerol. Furthermore, the elimination of acetone and 1,3-PDO due to the deficiency of *ctfA/B* and *dhaT* genes results in the unique BE pathway and relatively higher butanol ratio.

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Compliance with ethical standards

Conflict of interest The authors have declared there was no conflict of interest.

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