



## Effects of zinc on the production of alcohol by *Clostridium carboxidivorans* P7 using model syngas

Demao Li<sup>2</sup> · Chunxiao Meng<sup>1</sup> · Guanxun Wu<sup>1,2</sup> · Bintao Xie<sup>2</sup> · Yifan Han<sup>2</sup> · Yaqiong Guo<sup>2</sup> · Chunhui Song<sup>3</sup> · Zhengquan Gao<sup>1</sup> · Zhiyong Huang<sup>2,3</sup>

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### Abstract

Renewable energy, including biofuels such as ethanol and butanol from syngas bioconverted by *Clostridium carboxidivorans* P7, has been drawing extensive attention due to the fossil energy depletion and global eco-environmental issues. Effects of zinc on the growth and metabolites of *C. carboxidivorans* P7 were investigated with model syngas as the carbon source. The cell concentration was doubled, the ethanol content increased 3.02-fold and the butanol content increased 7.60-fold, the hexanol content increased 44.00-fold in the medium with 280  $\mu\text{M}$   $\text{Zn}^{2+}$ , when comparing with those in the control medium [ $\text{Zn}^{2+}$ , (7  $\mu\text{M}$ )]. Studies of the genes expression involved in the carbon fixation as well as acid and alcohol production in the medium with 280  $\mu\text{M}$   $\text{Zn}^{2+}$  indicated that *fdhII* was up-regulated on the second day, *acsA*, *fdhII*, *bdh35* and *bdh50* were up-regulated on the third day and *bdh35*, *acsB*, *fdhI*, *fdhIII*, *fdhIV*, *buk*, *bdh10*, *bdh35*, *bdh40* and *bdh50* were up-regulated on the fourth day. The results indicated that the increased  $\text{Zn}^{2+}$  content increased the alcohol production through increase in the gene expression of the carbon fixation and alcohol dehydrogenase.

**Keywords** *Clostridium carboxidivorans* P7 · Syngas fermentation · Zinc treatment · Alcohol synthesis · Gene expression

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Demao Li, Chunxiao Meng and Guanxun Wu contributed equally to this work.

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✉ Zhengquan Gao  
zq7723@126.com

✉ Zhiyong Huang  
huang\_zy@tib.cas.cn

<sup>1</sup> School of Life Sciences, Shandong University of Technology, Zibo 255000, Shandong, China

<sup>2</sup> Tianjin Key Laboratory for Industrial Biological Systems and Bioprocessing Engineering, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China

<sup>3</sup> Key Laboratory of Western China's Mineral Resources of Gansu Province, School of Earth Sciences, University of Lanzhou, Lanzhou 73000, Gansu, China

### Introduction

Currently, renewable energy has been drawing extensive attention due to the excess exploitation and use of fossil fuels, which not only threatens energy safety, but also causes serious environmental pollution [7, 35]. On the other hand, there are millions of tons of waste produced in the industrial society and in our daily life, such as stalk, food waste, waste gas etc. So, waste-to-energy was recognized as a good way to generate bioenergy along with solving the environmental problems.

Some industrial processes, such as oil refining [15], steelmaking and the production of carbon black, methanol and coke [16], gasification and pyrolysis [1, 17], will release a large amount of waste gases, and the main components are CO and H<sub>2</sub> (syngas). These waste gases can be used as raw materials for biological conversion into biofuels [30] through fermentation. Syngas fermentation is a process by which microorganisms use a mixture of H<sub>2</sub>, CO, and CO<sub>2</sub> to produce fuels and chemicals such as ethanol, butanol, hexanol, acetic acid, butyric acid, and methane [5, 20, 22, 29, 38]. The industrial utilization of syngas can accelerate the recovery of carbon resources and

decrease the dependence on fossil fuels. Syngas fermentation may also meet the strategic needs of the low-carbon development path. It not only has remarkable economic effects, but demonstrates significant ecological and environmental benefits. Therefore, the studies for syngas fermentation are particularly necessary.

A class of anaerobic microorganisms named acetogens can autotrophically use  $H_2 + CO_2$  or/and CO as carbon sources and can synthesize chemicals, such as ethanol, and butanol, making it an ideal catalyst for microbial syngas fermentation [13]. Acetogens use acetyl coenzyme A (acetyl-CoA) pathway, also known as the Wood–Ljungdahl pathway, to convert C1 substrates into acetyl-CoA. This approach is considered the oldest discovered carbon fixation pathway. This pathway uses an important  $CO_2/CO$  synthetic intermediate metabolite, acetyl-CoA, and CO or  $CO_2$  fixation can be achieved. This is accompanied by the transfer of energy during carbon fixation, whereas the carbon-immobilized product, acetyl-CoA, participates in the formation of the cytoskeleton [9]. The methyl and carbonyl groups obtained from the two branches of the Wood–Ljungdahl pathway bind to coenzyme A to produce an important intermediate, acetyl-CoA, which is further converted to organic acids and alcohols. Acetyl-CoA can be directly converted to acetic acid and ethanol, whereas butyric acid and butanol are directly produced from butyryl-CoA [11].

Well-studied acetogens in the laboratory and industry include *Butyribacterium methylotrophicum*, *Clostridium autoethanogenum*, *Clostridium carboxidivorans*, *Clostridium ljungdahlii*, *Clostridium ragsdalei*, and *Eubacterium limosum* [36]. Among these commonly used strains, *C. carboxidivorans* can use syngas to produce ethanol and higher alcohols, i.e., butanol and hexanol [6, 10]. However, biofuels from *C. carboxidivorans* P7 remains far from industrialization, which is attributed by reasons such as low gas–liquid mass transfer efficiency [12], difficulties in expanding the scale of fermentation, low rate of CO absorption and poor efficiency of conversion to alcohols [5, 37]. So far, efforts have been focused on solving these problems by increasing gas velocity, gas–liquid interface and gas solubility (by pressure and surfactant) to improve the efficiency of gas–liquid mass transfer [4]. In addition, domesticated strains with shortened fermentation times and improved CO tolerance will improve the fermentation efficiency.

Zinc is an essential nutrient for all the living organisms, which affects both cell growth and metabolism. Although zinc plays an important role in bacteria, high concentrations of zinc can have toxic effects on the bacteria. First, excess zinc ions compete with other metal ion-binding sites to inactivate the protein. Second, zinc can also form hydroxyl radicals, and cause damage to the DNA, protein and lipids. In addition, high concentrations of zinc ions directly inhibit the electron transport chain during electron transfer, causing

respiratory effects that cause damage [14]. Therefore, in bacteria, zinc ions must be tightly regulated [27].

There were several studies reported that suitable zinc supplementation in the culture medium would be beneficial for ethanol production [28, 42]. Zhao et al. [42] found that the ethanol tolerance, thermal tolerance and ethanol production were significantly improved by zinc supplements. However, no study on the gene expression changes in *C. carboxidivorans* P7 resulting from zinc supplementation along with ethanol fermentation was reported.

The effects of zinc stress on *C. carboxidivorans* P7 growth, CO uptake and chemicals production were studied in detail, as well as the expression levels of related genes were studied. This study will help to elucidate the effects and mechanism of zinc on bio-alcohols production and will provide a theoretical basis for the synthesis of bio-alcohols.

## Materials and methods

### Strain, medium and seed culture

*Clostridium carboxidivorans* P7 was a kind gift from the Professor Yang Gu. Laboratory stocks and they were maintained at  $-80\text{ }^\circ\text{C}$ . It was inoculated into the Wilkins Chalgren Anaerobe Broth, containing tryptone 10 g/L, fish peptone 10 g/L, yeast extract 5 g/L, glucose 2 g/L, NaCl 5 g/L, L-arginine 1 g/L, sodium pyruvate 1 g/L, L-cysteine 0.3 g/L (Adjust pH to 6.0 with solid NaOH), and 1 mL/L stock c (0.1 g  $NaHCO_3 + 100\text{ mL } H_2O$ ), 10 mL/L stock a (0.5 g of hemin + 10 mL 1 M NaOH + 990 mL  $H_2O$ ), and 200  $\mu\text{L/L}$  stock b (0.05 mL vitamin K1 solution + 20 mL 95% ethanol) [26] (filter sterilized, and medium components added after sterilization) [19, 26]. The inoculation ratio was 5% (v/v), and the seed culture was incubated upright in the anaerobic chamber at  $37\text{ }^\circ\text{C}$  for 36 h until exponential phase. Subculture was performed for approximately 12 h to achieve the exponential phase of cell growth. The gas fermentation medium contained NaCl 2.4 g/L,  $NH_4Cl$  3 g/L, KCl 0.3 g/L,  $KH_2PO_4$  0.3 g/L,  $MgSO_4$  0.6 g/L,  $CaCl_2$  0.12 g/L, L-cysteine-HCl 0.2 g/L, L-cysteine 0.2 g/L, yeast extract 0.5 g/L, MES 5 g/L, trace element solution (nitrilotriacetic acid 10 g/L,  $MnSO_4$  5 g/L,  $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$  4 g/L,  $CoCl_2 \cdot 6H_2O$  1 g/L,  $CuCl_2 \cdot 2H_2O$  0.1 g/L,  $NiCl_2 \cdot 6H_2O$  0.1 g/L,  $Na_2MoO_4 \cdot 2H_2O$  0.1 g/L,  $Na_2SeO_4$  0.1 g/L,  $Na_2WO_4 \cdot 2H_2O$  0.1 g) 2 mL/L, (adjust pH to 6.0 with NaOH), and 1 mL/L vitamin solution (VB<sub>6</sub> 0.1 g/L, thiamine 0.05 g/L, VB<sub>2</sub> 0.05 g/L, calcium pantothenate 0.05 g/L, thioacetic acid 0.05 g/L, p-aminobenzoic acid 0.05 g/L, nicotinic acid 0.05 g/L, VB<sub>12</sub> 0.05 g/L, biotin 0.02 g/L, and folic acid 0.02 g/L) (filter sterilized through a 0.22  $\mu\text{M}$  filter). The inoculation ratio was 5% (v/v). All the media were sterilized at  $121\text{ }^\circ\text{C}$  for 20 min, if there is no more statement of filter sterilization.

## Fermentation

To study the effect of  $Zn^{2+}$  on the syngas fermentation of strain P7, different concentrations of  $ZnSO_4 \cdot 7H_2O$  (0, 7, 70, 140, 280  $\mu M$ ) were added to the fermentation medium.

Serum bottles containing 20 mL of the fermentation media was inoculated with 5% (v/v) of *C. carboxidivorans* P7 seed culture. Artificial syngas (50%  $CO/35\% CO_2/15\% H_2$ ) was purged with a headspace pressure of 0.2 MPa after inoculation and every 24 h afterwards, and 1.5 mL samples were collected every 24 h before re-purge of the syngas in 4 days. The OD at 600 nm of 0.5 mL fermented broth was determined by a spectrophotometer (UV-1800) and expressed as  $OD_{600}$  for cell concentration. And 1 mL samples were centrifuged at 6000 rpm, 4 °C for 10 min. The supernatants were used to determine the content of alcohol and acid. And the pellet from the fermentation medium with 280  $\mu M$  of zinc as the experimental group and 7  $\mu M$  of zinc as the control group were transferred to –80 °C and kept for RNA extraction and Q-RT-PCR analysis.

## RNA extraction and cDNA synthesis

The extraction of *C. carboxidivorans* P7 total RNA was performed by using RNeasy Pure Cell/Bacteria Kit (TIANGEN BIOTECH, CN). Residual of DNA was evaluated by PCR with universal primers of bacteria 16s rRNA sequence [31]. First chain of cDNA was obtained by using FastQuant RT Super Mix (TIANGEN BIOTECH, CN) and stored at –20 °C.

## Gene expression detection via Q-RT-PCR

Quantitative real-time PCR (Q-RT-PCR) was performed for selected genes including carbon fixation, acidogenesis and solventogenesis pathways (supplemental materials S1) in different samples. The reactions were conducted with SYBR<sup>®</sup> Select Master Mix (Applied Biosystems, Austin, USA) for supporting the validity of the RNA-seq data. The LightCycler 96 real-time PCR system (Roche Diagnostics, Mannheim, Germany) was used to amplify and quantify the PCR of the products. The program was as follows: 5 min at 95 °C, followed by 40 amplification cycles of 10 s at 95 °C, 30 s at 60 °C. Transcript abundance of the target genes were normalized with the reference gene *guk* encoding guanine kinase [21]. Relative levels of transcript abundance of the studied genes were calculated with the  $2^{-\Delta\Delta CT}$  method [23]. Quantitative real-time PCR of each gene was performed with three reactions in parallel. The experiments were confirmed with biological triplicates.

## Determination of the products

Fermentation metabolites, including ethanol, butanol, hexanol, acetic acid, and butyric acid, were measured with an internal standard method using a gas chromatograph equipped with a flame ionization detector and capillary column (Zebron ZB-WaxPlus, Phenomenex, USA) [18, 41]. Samples from fermentation were prepared by centrifuging at 12,000×g for 5 min and filtrated with 0.22  $\mu m$  filters. The program was as follows: oven temperature 150 °C, injector temperature 180 °C, and detector temperature 180 °C. The internal standards were isobutanol, isobutyric acid and hydrochloric acid (acidified with 1 M HCl).

## Determination of CO utilization

The CO concentration ( $C_0$ ) was determined by gas chromatography with a TDX-01 80/100 column and thermal conductivity detector. The program was as follows: oven temperature 180 °C, detector temperature 150 °C, and injector temperature, 80 °C. Samples with known concentrations of CO were used to construct a standard curve.

The CO gas volume ( $V$ ) was determined using the drainage method at room temperature and atmospheric pressure. A 200 mL test tube full of water was upside down in a beaker full of water. And the gas in the serum bottle after fermentation was introduced to the test tube and the liquid surface of the test tube and serum bottles were kept at the same level until stable. The CO volume ( $V$ ) was calculated as the sum of the gas volume in the serum bottle and the gas volume in the test tube which was introduced from the serum bottles.

The total CO utilization was calculated as follows:

$$CO_{\text{utilization}} = \sum (C_0 * V). \quad (1)$$

The product conversion rate ( $P_{\text{rate}}$ ) was defined as the ratio of the maximum alcohol content and the CO utilization, as indicated by the following equation:

$$P_{\text{rate}} = c_{\text{max}} * (0.02 - 0.0015x_{\text{max}}) / M_{\text{max}}, \quad (2)$$

where  $c_{\text{max}}$ ,  $x_{\text{max}}$  and  $M_{\text{max}}$  are the maximum product yield, the time corresponding to the maximum yield of the product, and the total amount of CO absorbed at this time, respectively.

## Analysis of the data

All the experiments were conducted three times and the results presented were the mean values of the three samples and the standard deviation was showed.

## Results and discussion

### Effects of zinc on CO utilization and product yield by *C. carboxidivorans* P7

The cell concentration (Fig. 1), CO utilization (Fig. 2a), product yield (Fig. 2b, c) and CO conversion rate (Fig. 2d) of *C. carboxidivorans* P7 in different zinc concentrations have been studied. The cell concentration, product yield and CO conversion rate of *C. carboxidivorans* P7 improved by increasing the zinc concentration. The cell concentration, ethanol production, butanol production, hexanol production and total CO conversion rate in medium with 280  $\mu\text{M}$   $\text{Zn}^{2+}$  increased 1.03 times, 3.02 times, 7.60 times,

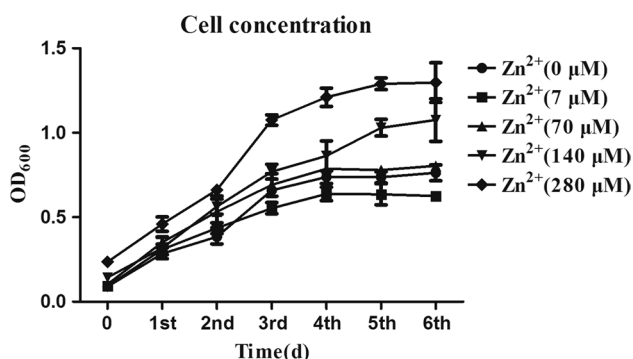


Fig. 1 The effects of zinc on cell growth expressed as OD<sub>600</sub>

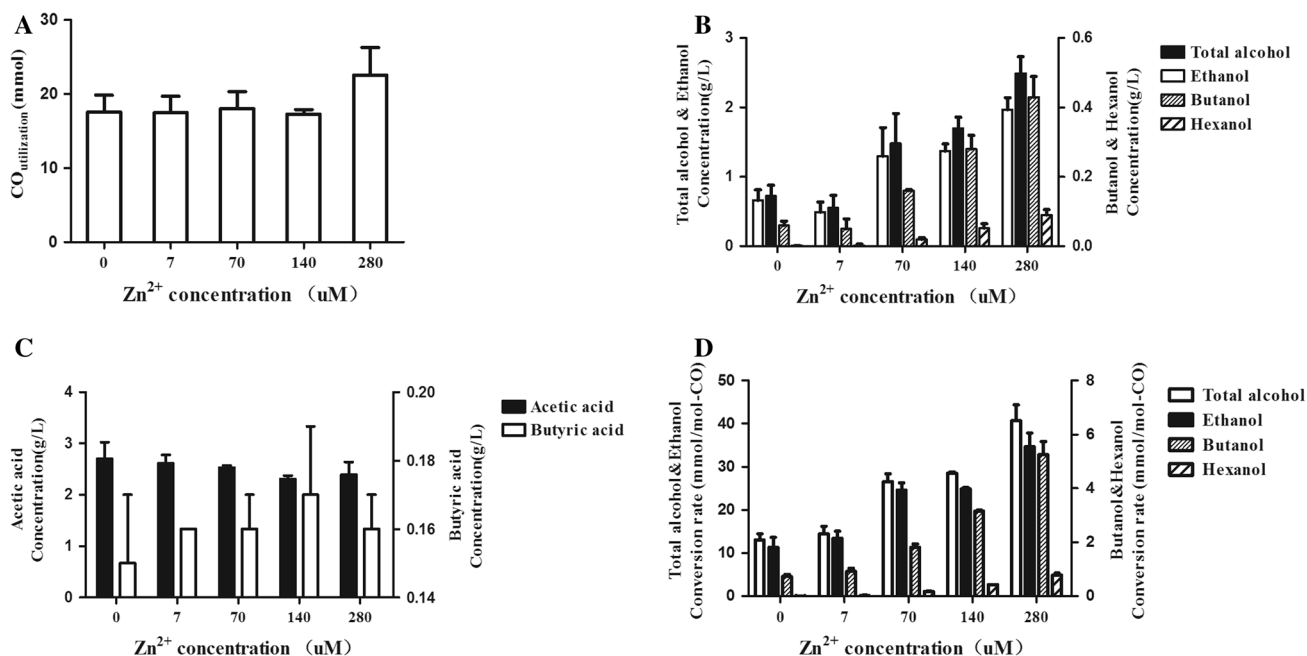


Fig. 2 The effects of zinc on CO<sub>utilization</sub> (a), alcohol production (b), acid production (c),  $P_{rate}$  (d)

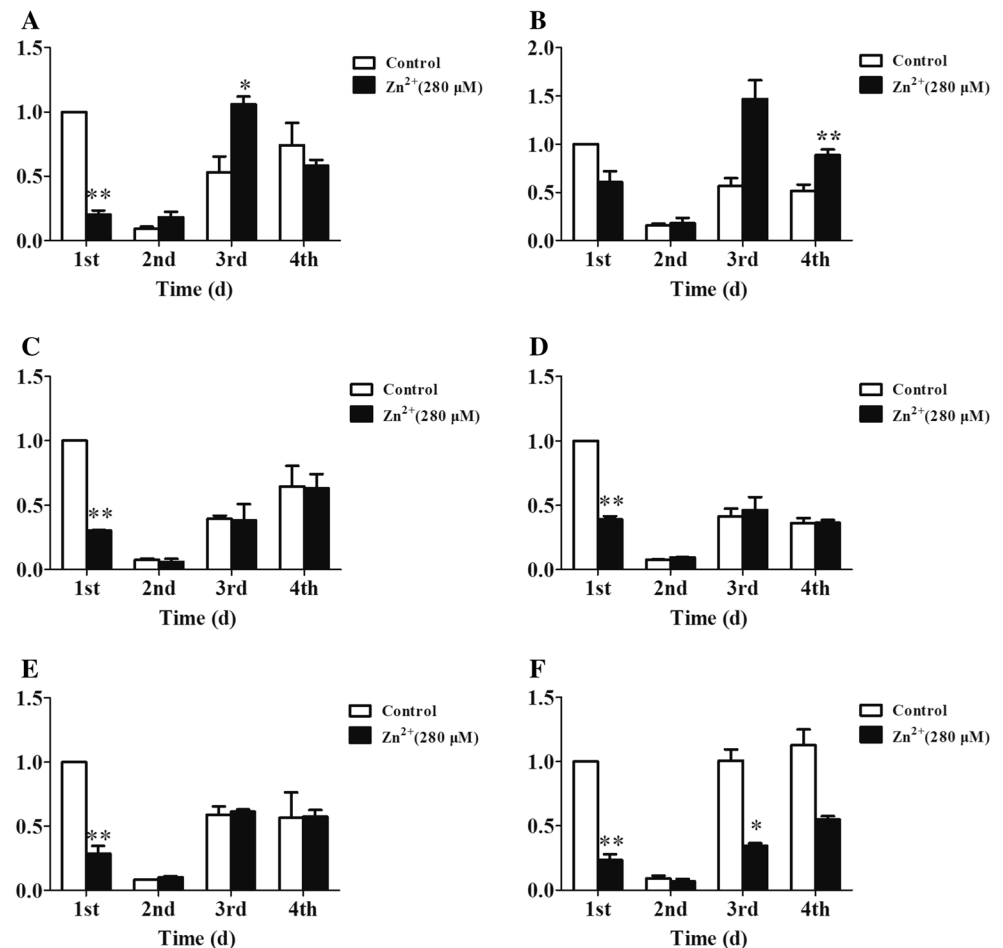
44.00 times and 1.83 times, when compared with the control medium with 7  $\mu\text{M}$   $\text{Zn}^{2+}$ .

Similar results was also found in *C. acetobutylicum*. Production of acetone, ethanol, and butanol was improved with zinc supplementary, which was attributed to the rapid acid's re-assimilation for more efficient ABE production [39].

### Effects of zinc on gene expression of *C. carboxidivorans* P7

Figure 3 shows the gene expression of *C. carboxidivorans* P7 metabolic pathway associated with the CO dehydrogenase-related gene, in which expression of gene *cooCII* was not detected. The CO-related genes were significantly down-regulated ( $P < 0.01$ ) in medium containing 280  $\mu\text{M}$   $\text{Zn}^{2+}$  compared with control medium, except for the *acsB* gene (Fig. 3a, c, f). In the early growth phase (the 1st day), the effect of  $\text{Zn}^{2+}$  was not obvious in the low cell concentration (Fig. 1). The next day, the expression levels of all the genes decreased ( $P < 0.05$ ), and this trend was likely due to the depletion of glucose which was carried in by inoculation and was adapting to the carbon source of CO [3]. On the 3rd day, the expression levels of all the genes increased, and the expression of the *acsA* gene (Fig. 3a) was significantly up-regulated in the high  $\text{Zn}^{2+}$  treatment ( $P < 0.05$ ). However, the expression of the *cooCII* (Fig. 3f) gene was significantly down-regulated ( $P < 0.05$ ). Until the 4th day, CO-related gene (*acsA*, *acsD*, *acsE*) expression decreased significantly, but the expression of the *acsB* gene (Fig. 3b) was significantly up-regulated in medium with 280  $\mu\text{M}$

**Fig. 3** The gene expression of *acsA* (a), *acsB* (b), *acsC* (c), *acsD* (d), *acsE* (e) *cooI* (f). Data in bold indicate significant differences, \* $P < 0.05$ , \*\* $P < 0.01$



Zn<sup>2+</sup> ( $P < 0.01$ ). In general, changes in the expression levels of the CO-related genes under different zinc treatments were similar to those of the control group. The significant increase of the *acsA* gene on the 3rd day and *acsB* gene expression content on the 4th day were the main reasons for the improvement of CO utilization (Fig. 3a, b).

Figure 6 shows the relative expression of the formate dehydrogenase-related gene in the *C. carboxidivorans* P7 metabolic pathway. The formate dehydrogenase-related genes under different Zn<sup>2+</sup> treatments of the 1st day were significantly inhibited ( $P < 0.05$ ). The expression of the *fdhII* (Fig. 4b) gene was significantly increased ( $P < 0.05$ ) on the 2nd day, and the other genes, such as *fdhI*, *fdhIII*, *fdhIV*, and *fdhV* (Fig. 4a, c–e) did not change significantly ( $P > 0.05$ ) in the high Zn<sup>2+</sup> treatment, when comparing to that with the control treatment. On the 3rd day of the cultivation, only the expression of *fdhI* and *fdhII* (Fig. 4a, b) increased significantly ( $P < 0.05$ ). On the 4th day, the expression of *fdhIII* and *fdhIV* (Fig. 4c, d) were significantly up-regulated ( $P < 0.05$ ), and among them, *fdhIII* (Fig. 4c) was very significant ( $P < 0.01$ ). The results indicated that the gene for formate dehydrogenases up-regulated was expressed under the high Zn<sup>2+</sup> treatment. Thus, the high Zn<sup>2+</sup> treatment has

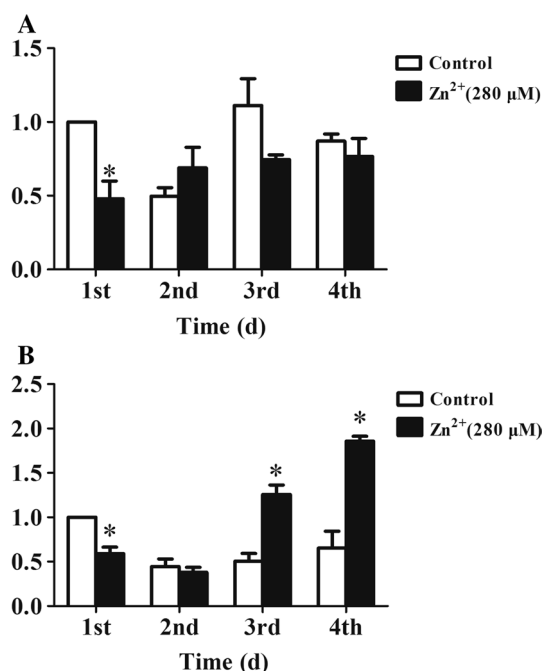
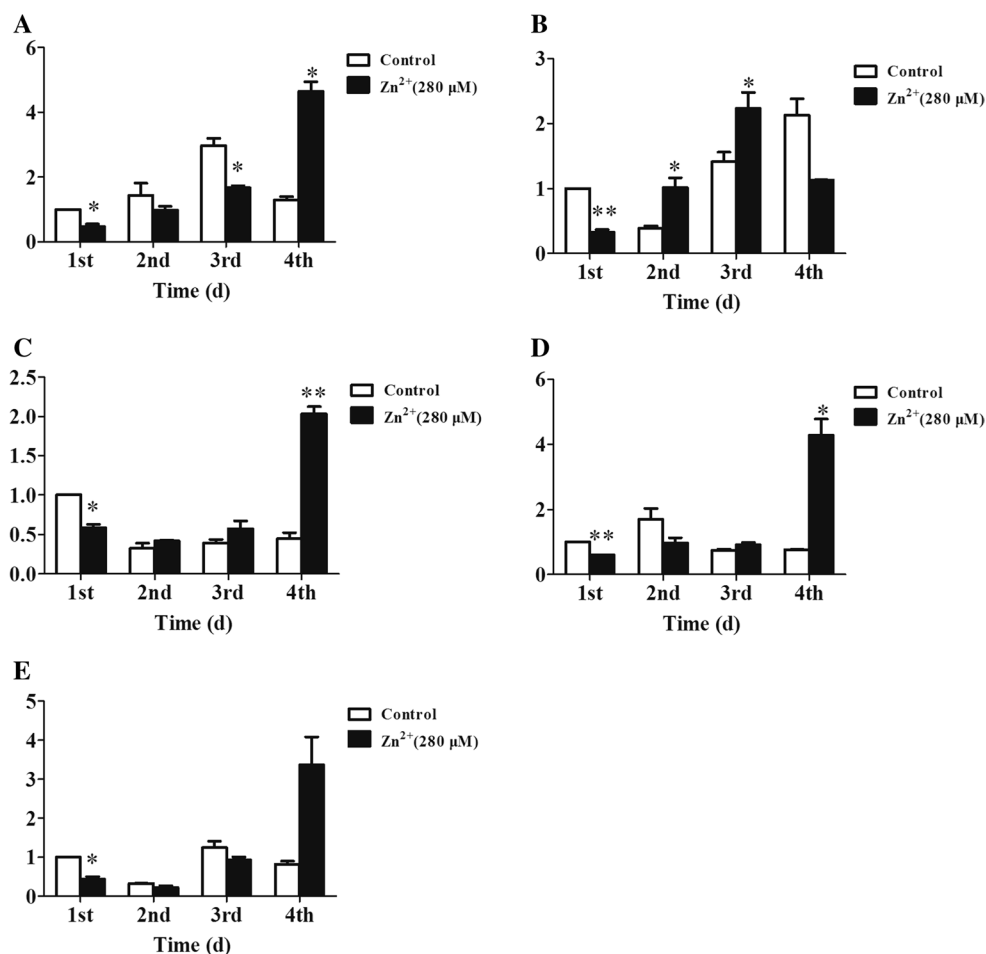
a sustained stimulating effect on the formic acid dehydrogenase gene, whereas the control group gene expression trend did not have a obvious change during the studies.

FDH is the key enzyme for the reduction of CO<sub>2</sub> to formate in *C. formicoaceticum* and *C. thermoaceticum* [2, 40]. Five types of FDH have been found in *C. carboxidivorans* P7 and most of them were up-regulated, and then attributed to the capacity for carbon fixation.

Figure 5 shows the relative expression of the acetic acid/butyric acid kinase-related gene in the *C. carboxidivorans* P7 metabolic pathway. The trend for the acetic acid kinase gene expression of the control group first increased, whereas high Zn<sup>2+</sup> treatments were basically unchanged (Fig. 5a). Expression of the acetic acid kinase gene was significantly down-regulated on the 1st day compared to the control group ( $P < 0.05$ ). The expression of the butyric acid kinase gene (Fig. 5b) in the control group was stable. However, it increased under the high zinc treatment from the 2nd to 4th day, except the significant decrease on the 1st day ( $P < 0.05$ ). The relative expression of the gene on the 3rd day and the 4th day was significantly increased ( $P < 0.05$ ).

More ATPs can be produced during the organic acid formation comparing with that during alcohol formation,

**Fig. 4** The gene expression of formate dehydrogenase *fdhI* (a), *fdhII* (b), *fdhIII* (c), *fdhIV* (d); *fdhV* (e). Data in bold indicate significant differences, \* $P < 0.05$ , \*\* $P < 0.01$

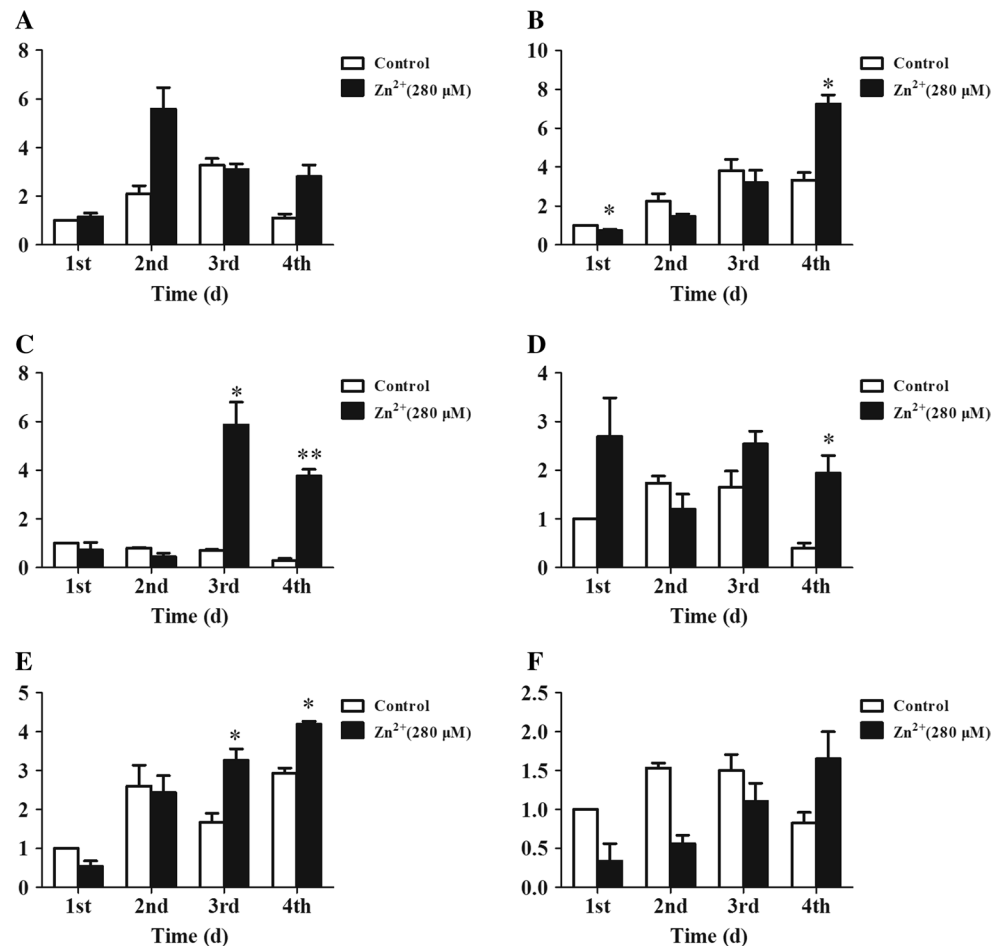


**Fig. 5** The gene expression of *ack* (a), *buk* (b). Data in bold indicate significant differences, \* $P < 0.05$ , \*\* $P < 0.01$

which will be helpful for cell growth [33]. The acetic acid kinase gene expression did not change after zinc supplementation, which was verified by the acetic acid production. The expression of the butyric acid kinase gene was up-regulated than that of the control, however, the butyric acid production did not change during cultivation significantly. This may be caused by the up-regulation of the gene expression that did not lead to the up-regulation of the butyric acid kinase activity, or there may be more butyric acid produced which was utilized for butanol production.

Figure 6 shows the relative expression of the alcohol dehydrogenase and acetyl-CoA transferase-related genes in *C. carboxidivorans* P7 metabolic pathway. The alcohol dehydrogenase genes (Fig. 6a) in the control group and experimental group have changed similarly, first increasing and then decreasing. The relative expression of acetyl-CoA transferase (Fig. 6f) did not change significantly, but gene expression under high zinc treatment showed an increasing trend. The genes *bdh10*, *bdh35*, *bdh40*, *bdh50* (Fig. 6b–e) related to butanol dehydrogenase except *bdh10* were significantly lower on the 1st day for the high zinc treatment, when compared with the control group ( $P < 0.05$ ), and the others showed no significant change in the first 2 days. The

**Fig. 6** The gene expression of *adh* (a), *bdh10* (b), *bdh35* (c), *bdh40* (d), *bdh50* (e), *CoAT* (f). Data in bold indicate significant differences, \* $P < 0.05$ , \*\* $P < 0.01$



expression levels of *bdh35* (Fig. 6c) and *bdh50* (Fig. 6e) were up-regulated on the 3rd day and 4th day, and the expression of the butanol dehydrogenase gene was significantly up-regulated on the 4th day, compared with the control group ( $P < 0.05$ ). In particular, the *bdh35* gene (Fig. 6c) was significantly different on the 4th day ( $P < 0.05$ ).

Alcohol dehydrogenase (Fig. 6a) and acetyl coenzyme A transferase gene (Fig. 6f) expression levels did not change significantly, compared with the control group, whereas the production increased. The synthesis of ethanol and the acetic acid precursor acetyl-CoA content were significantly increased, comparing with that of the control group, suggesting that carbon fixation was improved under the high zinc treatment.

In general, high zinc levels led to delayed growth on the 1st day, however, most of the metabolic processes and gene expression levels were significantly higher than in the control on the following days, indicating increased fermentation activity.

A large number of protein constituents are active in the presence of zinc, in which some of the zinc is involved in the active center of the cell signaling pathway that constitutes the transcription factor [34]. The zinc supplementation is

beneficial to the production of ethanol, but the mechanism of high production is different in different references [8, 24, 32].

Studies considered that zinc not only contributes to the rapid use of glucose and increase the cell growth, but also promotes the re-assimilation of acid and butanol production, and promotes the initiation of *C. acetobutylicum* solvent generation [39]. They found that zinc has a significant impact on the central carbon metabolism of hydrazine, *aad*, *adhE2*, *cac3292*, *cap0059*, *thLB* and other genes, suggesting that zinc can significantly enhance the acetone butanol process, thereby promoting acid generation and solvent generation [39].

Our results indicated that zinc supplementation stimulated cell growth and alcohol production. The quantitative fluorescence analysis results indicated that zinc supplementation also stimulated most of the metabolic fermentation pathways of the genes, such as *acsA* (Fig. 3a), *acsB* (Fig. 3b), *fdhI* (Fig. 4a), *fdhII* (Fig. 4b), *fdhIII* (Fig. 4c), *fdhIV* (Fig. 4d), *buk* (Fig. 5b), *bdh10* (Fig. 6b), *bdh35* (Fig. 6c), *bdh40* (Fig. 6d) and *bdh50* (Fig. 6e), thus affecting the synthesis of alcohol.

Zinc supplementation increased the product yield maybe due to the promotion of carbon fixation. From the genetic level, the expression of the alcohol dehydrogenase gene (Fig. 6a), the acetyl-CoA transferase gene (Fig. 6f) and acetate kinase (Fig. 5a) were not increased significantly by zinc supplementation. However, it stimulated the ethanol production (Fig. 2b). Zinc can increase the amount of acetyl-CoA, and the increase in acetyl-CoA is due to carbon fixation. The increase of CO absorption and the increase of the conversion rate indicated that zinc supplementation promotes the uptake and transformation of CO, leading to an increase of acetyl-CoA content in the precursor of synthetic ethanol, which eventually leads to the increase of ethanol content.

Zinc is a key factor affecting the biological function of butanol dehydrogenase, which is a key enzyme responsible for the synthesis of butanol [39]. The results indicated that zinc supplementation also stimulated the expression of butanol dehydrogenase gene (Fig. 6b–e) and butyrate kinase gene (Fig. 5b). Thus, butyric acid, whose production was increased by the increased expression of butyrate kinase gene, was converted to butanol. Then, the increased ratio of butanol was higher than that of ethanol.

Zinc supplementation maybe double-edged, i.e., some enzyme activities can be improved, but some maybe inhibited [25]. We found that after adding zinc, most of the genes on the 1st day are inhibited to varying degrees, and the need for an adaptive process of gene expression can be restored to normal levels and even significantly increased.

These results indicated that zinc supplementation contributes to carbon fixation and the synthesis of alcohol-related gene expression. Zinc supplementation can change the ratio of acid and alcohol in the fermentation broth; therefore, it is important for increasing the substrate utilization, reducing production costs, and improving biofuel production.

## Conclusions

Through the combinational analysis of CO concentration, fermentation metabolites and gene expression, we demonstrated for the first time that, zinc regulates the de novo synthesis of alcohols rather than acid re-assimilation in this strain. This knowledge helps fine-tune the medium composition to improve alcohol production, and also identifies potential genetic targets for future bio-engineering to optimize the C1-based bio-chemical synthesizing platform.

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