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ORIGINAL ARTICLE

Impact of deletion of the genes encoding acetate kinase on production of L-tryptophan by *Escherichia coli*

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Abstract The production of L-tryptophan was increased by reducing acetate accumulation through a decrease in acetate kinase activity by gene deletion. The effects of disruption of the genes for acetate kinase (*ackA*) and an enzyme with propionate/acetate kinase activity (*tdcD*) on L-tryptophan production were investigated. The *ackA* and/or *tdcD* deletion mutants accumulated less acetate and more L-tryptophan than the parental strain. Furthermore, the production of L-tryptophan obtained with *ackA-tdcD* mutant were more than the mutants with a single deletion of *ackA* or *tdcD*, while higher production of L-tryptophan and lower concentration of acetate were accumulated in the *ackA* mutant than the mutant with a lesion in *tdcD*. In L-tryptophan fed-batch fermentation using the *ackA-tdcD* mutant, the excretion of acetate was reduced to 1.22 g/L, a 21.79 % reduction compared with the parental strain, and the production of L-tryptophan and glucose conversion rate were increased to 52.5 g/L and 47.9 g/L, respectively, which represented 6.49 % and 10.88 % increases compared with the parental strain, and the glucose conversion rate reached a high level of 21.2 %, which was 8.16 % higher than the parental strain. In addition, the metabolic flux analysis of TRTH and TRTHAT indicated that the carbon flux through EMP was decreased by 8.37 % and the carbon flux through PP was increased by 57.03 % in TRTHAT compared with TRTH. The flux of acetate and tryptophan formation of TRTHAT were 5.2 % and 17.3 %, which were 3.67-times lower and 1.75-times higher than these of TRTH, respectively.

Keywords *Escherichia coli* · L-Tryptophan · Acetate kinase · *ackA* · *tdcD* · Metabolic flux

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Introduction

As an essential amino acid for humans and other animals, L-tryptophan is widely supplemented in food, animal feed, and medicine (Liu et al. 2012). As the third most limited amino acid, L-tryptophan is widely added to common feedstocks (Zhao et al. 2011; Liu et al. 2012). However, the market for L-tryptophan is still limited because of its rather high production costs (Ikeda 2006). Because of its commercial importance, optimization of L-tryptophan production is of interest with the aim to decrease its production costs (Liu et al. 2012). L-tryptophan can be obtained from cheap and renewable carbon sources such as sucrose or glucose by microbial fermentation (Ikeda 2006), in which *Escherichia coli* is used as the preferred L-tryptophan-producing strain. The production of L-tryptophan has been improved by genetically modifying *E. coli*, including overexpression of key genes for tryptophan biosynthesis, disruption of some important genes that repress tryptophan formation, and modification of the tryptophan transport system (Gu et al. 2012; Shen et al. 2012; Liu et al. 2012; Wang et al. 2013a).

The excretion and extracellular accumulation of acetate, the primary inhibitory metabolite in cultures of *E. coli*, is a common problem encountered in high-cell-density cultivation

of this bacterium (De Anda et al. 2006). The accumulation of acetate inhibits cell growth and impairs the capacity of *E. coli* to produce the desired product (Gosset 2005), and there are examples where the production of the desired product was significantly improved by decreasing the accumulation of acetate (Eiteman and Altman 2006). The manipulation of the culture environment to maintain either a low glucose concentration or eliminate the accumulated acetate as well as the application of metabolic pathway engineering to reduce carbon flow to the acetate-producing pathways have been applied to reduce the excretion of acetate in *E. coli* cultures (De Mey et al. 2007; Martínez-Gómez et al. 2012). In *E. coli*, the accumulation of acetate was decreased by maintaining the concentration of glucose at a low level with a combined feeding strategy, leading to increased biomass and a higher yield of L-tryptophan (San et al. 2002; Cheng et al. 2012).

The conversion of acetyl-CoA through the action of phosphotransacetylase (*pta*) and *ackA* and the conversion of pyruvate directly into acetate via pyruvate oxidase B (*poxB*) contribute to acetate formation (Phue and Shiloach 2005). The elimination of Pta and AckA activities has resulted in a significant reduction in acetate accumulation (De Anda et al. 2006). The Δ*pta* mutant presented lower production and production rate of acetate than wild-type *E. coli* (Chang et al. 1999). It has also been observed that, when *pta* is deleted in *E. coli*, pyruvate accumulates in the cell because of the complete alternation of the fermentation profile (Zhu and Shimizu 2005). We previously constructed a recombinant *E. coli* with a lesion in *pta* used for producing L-tryptophan, and the results indicated that the excretion of acetate was decreased and production of L-tryptophan was increased compared with the parental strain (Huang et al. 2011; Wang et al. 2013a, b), but the effect of the deletion of the genes encoding acetate kinase on L-tryptophan production has not so far been studied.

It has been reported that a proportion of the acetate could also originate from propionyl-CoA via acrylyl-CoA, lactyl-CoA and pyruvate intermediates, which is in accordance with the existence of another route to acetate synthesis (Heßlinger et al. 1998). The enzymes TdcE and TdcD are also known to play an important role in the formation of acetate. TdcE has both pyruvate formate-lyase and 2-ketobutyrate formate-lyase activities, whereas the TdcD protein is a new propionate/acetate kinase (Zhou et al. 2011). TdcD exhibits a high identity with acetate kinase from *E. coli* (Grundy et al. 1993). TdcD has propionate and acetate kinase activities, and overproduction of TdcD results in a 38-fold increase in acetate kinase-specific enzyme activity (Heßlinger et al. 1998). In addition, a lower concentration of acetate was accumulated in the *ackA-tdcD* double mutant compared with the mutant with the single deletion of *ackA* (Kumari et al. 1995).

The utilization of the metabolic genes is dependent on specific environmental parameters, including carbon source and the substrate availability, and the metabolic behavior of strain is affected by the gene deletion (Edwards and Palsson 2000). The deletion of *pta* and *ackAB* impacted the production capabilities of several of the biosynthetic precursors, such as glucose 6-phosphate (Glc6P), fructose 6-phosphate (F6P), ribose 5-phosphate (R5P), erythrose 5-phosphate and glyceraldehyde 3-phosphate (Edwards and Palsson 2000). For L-tryptophan production by *E. coli*, the disruption of *pta* decreased the metabolic flux of the Embden-Meyerhof-Parnas (EMP) pathway and the tricarboxylic acid (TCA) cycle and increased the metabolic flux of the pentose phosphate (PP) pathway and tryptophan synthesis, leading to obtaining lower accumulation of acetate and higher production of L-tryptophan (Huang et al. 2011).

In this study, the activity of acetate kinase was reduced by disruption of the genes *ackA* and *tdcD*, and three recombinant *E. coli* strains were generated from TRTH (*trpEDCBA*+Tet^R, Δ*tna*): TRTHA (TRTH, Δ*ackA*), TRTHT (TRTH, Δ*tdcD*) and TRTHAT (TRTH, Δ*ackA* Δ*tdcD*). Tryptophan production by these strains was carried out in a 30-L fermentor using the combined feeding strategy of pseudo-exponential and glucose-stat feeding to investigate the effect of deletion of *ackA* and/or *tdcD* on the production of L-tryptophan. Meanwhile, the metabolic flux distribution of TRTH and TRTHAT were analyzed to study the principle of lower acetate accumulation and higher L-tryptophan production obtained in the mutant with lesions in *ackA* and *tdcD*.

Materials and methods

Bacterial strains, plasmids, and primers

All bacterial strains, plasmids, and primers are listed in Table 1.

Media

The media used for generating and propagating the recombinant strains were prepared according to published procedures (Liu et al. 2012; Wang et al. 2013a). The seed medium consisted of 20 g/L glucose, 15 g/L yeast extract, 10 g/L $(NH_4)_2SO_4$, 0.5 g/L sodium citrate, 5 g/L $MgSO_4 \cdot 7H_2O$, 1.5 g/L KH_2PO_4, 0.015 g/L $FeSO_4 \cdot 7H_2O$, and 0.1 g/L vitamin B_1. The fermentation medium for producing L-tryptophan consisted of 10 g/L glucose, 1 g/L yeast extract, 4 g/L $(NH_4)_2SO_4$, 2 g/L sodium citrate, 5 g/L $MgSO_4 \cdot 7H_2O$, 2 g/L KH_2PO_4, and 0.1 g/L $FeSO_4 \cdot 7H_2O$. The pH of the seed and fermentation media were adjusted to 7.0 using 4 mol/L NaOH.

Culture conditions

Culture conditions used for generating and propagating recombinant strains were controlled according to published

Table 1 Strains, plasmids, and primers used in this study

Name	Characteristics	Source
Strains		
TRTH	*trpEDCBA*+Tet^R, Δ*tna*	Liu et al. 2012
TRTHA	Derived from TRTH, but Δ*ackA*	This study
TRTHT	Derived from TRTH, but Δ*tdcD*	This study
TRTHAT	Derived from TRTH, but Δ*ackA*, Δ*tdcD*	This study
Plasmids		
pKD46	Am^R, λ Red-expressing vector	Cherepanov and Wackernagel 1995
pKD3	Cm^R, Template vector	Cherepanov and Wackernagel 1995
pCP20	Am^R, Cm^R, FLP-expressing vector	Cherepanov and Wackernagel 1995
Primers		
ackA-P1	5'-CTGTCCCCGGCGAAACAAGCTAAAAAAATTAACAGAACGATTATCCGGCGTTGACATTGAGCGATTGTGT AGGCTGGAG-3'[b]	This study
ackA-P2	5'-CGGATCACGCCAAGGCTGACGCTGGTCAGACCGACGCTGGTTCCGGTAGGGATCAGTAACGGCTGACATGGGAATTAGC-3'[b]	This study
ackA-P3	5'-TGCCCAGCCACCACAATC-3'	This study
ackA-P4	5'-GTGGTAGTTTGCGACGAT-3'	This study
tdcD-P1	5'-GTGGGAGAGATCTCACTAAAAACTGGGGATACGCCTTAAATGGCGAAGAAACGGTTTGAGCGATTGTGTAGGCTGGAG-3'[b]	This study
tdcD-P2	5'-CATCCTGAACATCGTATACAAACTGTTTTAATCCGTAACTCAGGATGAGAAAAGAGTAACGGCTGACATGGGAATTAGC-3'[b]	This study
tdcD-P3	5'-CGGGCGGACCAAATGATAC-3'	This study
tdcD-P4	5'-AACCCGAACATCCTTGAC-3'	This study

[a] The TRTH was stored at the Culture Collection of Tianjin University of Science and Technology under the collection number TCCC 27003

[b] The underlined portions indicate 56-nt homology extensions of the target knockout gene

procedures (Liu et al. 2012). A 500-mL baffled flask containing 30 mL seed medium was inoculated with a single colony of each test strain, and cultivated at 36 °C with shaking at 200 rpm for 12 h. A 30-mL inoculum of this culture was added aseptically to a 5-L seed fermentor containing 3 L seed medium, and cultivated at 36 °C for 16 h. The culture grown in the seed fermentor was inoculated aseptically (10 % v/v) into 18 L of production medium in a 30-L fermentor. The temperature and dissolved oxygen level were maintained at 36 °C and 20 %, respectively. To maintain the pH at 7.0, 25 % ammonium hydroxide (w/w) was used. When the initial glucose was depleted, glucose solution (80 % w/v) was fed into the fermentor according to the combined feeding involving pseudo-exponential and glucose-stat feeding (Cheng et al. 2012).

Construction of the strains

Genes knockout mutants were constructed as described previously (Datsenko and Wanner 2000; Wang et al. 2013a). Disruption of *ackA* in TRTH was performed using the Red helper plasmid, pKD46. The appropriate DNA fragment was obtained by polymerase chain reaction (PCR) using the primers *ackA*-P1 and *ackA*-P2 with the helper plasmid pKD3. To eliminate the Cm^R gene from the integrated locus, the cells were transformed with the plasmid pCP20 carrying the FLP recombinase gene. All test PCRs were used with the primers *ackA*-P3 and *ackA*-P4. Disruption of *tdcD* in TRTH and TRTHA were performed using the same method as described for disrupting *ackA* using the primers *tdcD*-P1, *tdcD*-P2, *tdcD*-P3, and *tdcD*-P4.

Analysis of fermentation products

The dry cell weight (DCW) and concentration of L-tryptophan in the fermentation broth were determined as described previously (Cheng et al. 2012; Wang et al. 2013a). The concentrations of glucose and lactate were monitored using an SBA-40C biosensor analyzer (Biology Institute of Shandong Academy of Sciences, Jinan, China). Concentrations of acetate and pyruvate were measured using a Bioprofile 300A biochemical analyzer (Nova Biomedical, Waltham, MA, USA).

Analysis of kinetics data

According to the DCW and Logistic equation, a model of cell growth kinetics was constructed using Originpro 8.0 (Zawada and Swartz 2005; Hajji et al. 2007). The production rate of L-tryptophan and acetate, glucose consumption and conversion rate were calculated as described previously (Wang et al. 2013a).

Analysis of metabolic flux

Based on the analysis of metabolic flux balance and stoichiometry model, the distribution of metabolic flux with different strains in L-tryptophan production were calculated by MATLAB (Edwards and Palsson 2000; Schmid et al. 2004; Huang et al. 2011).

Statistical analysis

All experiments were conducted in triplicate, and the data were averaged and presented as the mean±standard deviation. One-way analysis of variance followed by Dunnett's multiple comparison test were used to determine significant differences. Statistical significance was defined as $p<0.05$.

Results

Recombinant strains

Colony PCRs using the primers *ackA*-P3-*ackA*-P4 and *tdcD*-P3-*tdcD*-P4 were used to confirm the knockouts of *ackA* or *tdcD*, respectively. The lengths of the fragments detected agreed with their theoretical lengths. Thus, the strains with deletions in *ackA* and/or *tdcD* were obtained.

DCW and growth rate of strain

The DCW and specific growth rate of the parental strain and mutants are presented in Fig. 1. The results indicated that the deletion of *ackA* and/or *tdcD* reduced the specific growth rate during the early stage of L-tryptophan fermentation and increased the specific growth rate during the later period of L-tryptophan fermentation according to the analysis of the cell growth kinetics model. The DCW of the parental strain was higher than that of the mutants during the early fermentation stage, but at the end of L-tryptophan production, the DCW obtained in TRTHA, TRTHT, and TRTHAT were increased by 4.87, 2.64, and 6.49 %, respectively, compared with that of the parental strain (49.3 g/L). In cultures of TRTHAT, the specific growth rate during the initial fermentation period was the lowest and that during the later fermentation period was the highest, and the maximum DCW (52.5 g/L) was obtained.

Production and production rate of L-tryptophan

The production and production rate of L-tryptophan in L-tryptophan production using the parental strain and mutants are displayed in Fig. 2. The L-tryptophan production of the mutants were higher than that of the parental strain, and the L-tryptophan production obtained in the mutant with a lesion in *ackA-tdcD* were higher than the mutants with the single deletion of *ackA* or *tdcD* and the L-tryptophan production of TRTHT was lower than that of TRTHA. The L-tryptophan production rate of the mutants were higher than that of the parental strain. The L-tryptophan production of TRTHAT (47.9 g/L) was 10.88 % higher than that of the parental strain (43.2 g/L).

Accumulation of acetate

The concentration of acetate with the parental strain and the mutants are presented in Fig. 3, along with its production/consumption rate. The results showed that the accumulation of acetate and its production/consumption rate were decreased in cultures of the mutants, and the production/consumption rate of acetate was decreased with reducing the excretion of acetate. The acetate accumulation of TRTHAT was lower than that of TRTHA and TRTHT, and the concentration of acetate accumulated in TRTHA was lower than the TRTHT. In cultures of TRTHAT, the acetate was accumulated to a

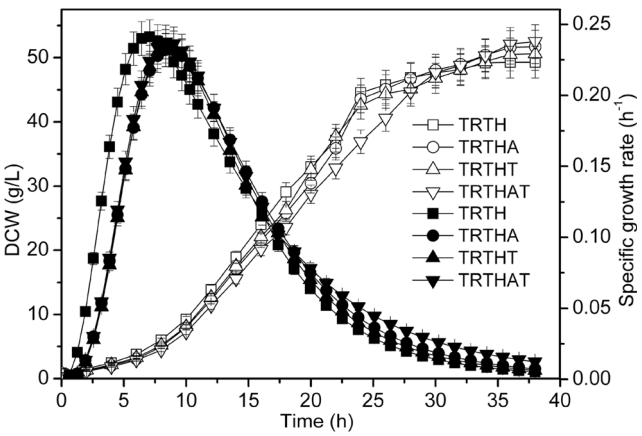

Fig. 1 Effect of gene modification on DCW and specific growth rate in L-tryptophan production. *Open symbols* DCW, *filled symbols* specific growth rate ($p<0.05$)

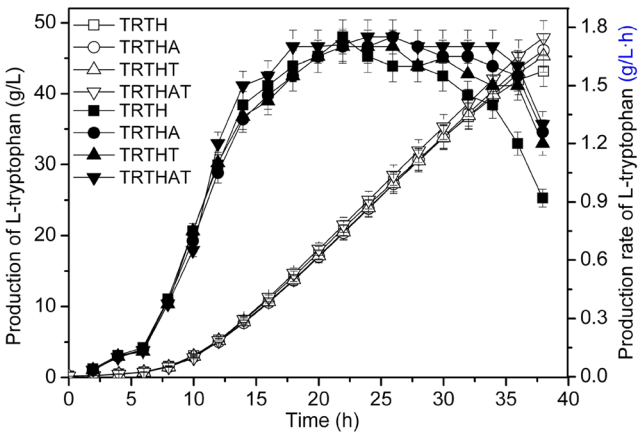

Fig. 2 Effect of gene modification on production and production rate of L-tryptophan in L-tryptophan production. *Open symbols* production of L-tryptophan, *filled symbols* production rate of L-tryptophan ($p<0.05$)

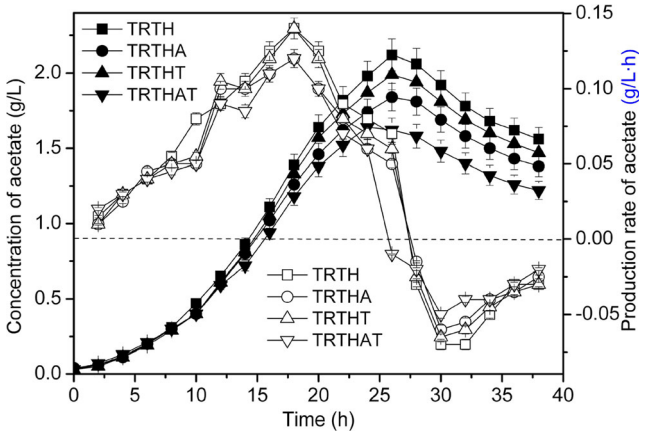

Fig. 3 Effect of gene modification on accumulation and production rate of acetate in L-tryptophan production. *Filled symbols* accumulation of acetate, *open symbols* production rate of acetate. The absolute value of the data less than zero indicates the consumption rate of acetate ($p<0.05$)

concentration of 1.22 g/L which was decreased by 21.79 % compared with the parental strain.

Excretion of pyruvate and lactate

Figure 4 shows the concentrations of pyruvate and lactate for each strain from L-tryptophan production. The concentrations of pyruvate and lactate obtained in the mutants were higher than these in the parental strain, and pyruvate and lactate accumulated during strain growth were consumed by the cells during the later fermentation period, leading to a decrease of their concentrations. The concentrations of pyruvate (0.23 g/L) and lactate (2.24 g/L) accumulated in the cultures of TRTHAT during the growth phase were highest, 1.31- and 1.19-times higher than these of the parental strain, respectively. The strains with mutations in *ackA* or *tdcD* excreted lower concentrations of pyruvate and lactate than these of TRTHAT, and 0.21 g/L pyruvate and 2.12 g/L lactate were accumulated in the cultures of TRTHA, which were increased by 5.04 % and 5.56 % compared with the TRTHT. Furthermore, pyruvate and lactate of each strain were totally consumed at the end of L-tryptophan fermentation.

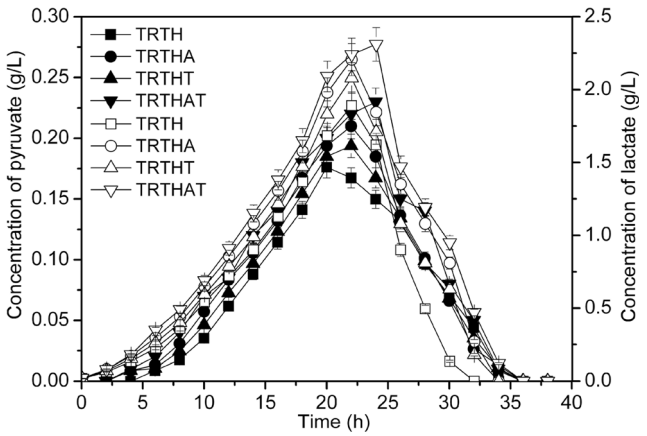

Fig. 4 Effect of gene modification on concentrations of pyruvate and lactate in L-tryptophan production. *Filled symbols* concentration of pyruvate, *open symbols* concentration of lactate ($p<0.05$)

Glucose consumption and conversion rate

The glucose consumption and conversion rate for each strain in L-tryptophan production are displayed in Fig. 5. During the early fermentation period, both the consumption rate of glucose and the glucose conversion rate were increased with the strain growth and L-tryptophan formation. In the stationary phase, glucose was chiefly used for L-tryptophan biosynthesis, leading to a high level of glucose conversion rate, while the rate of glucose consumption was decreased. At the end of L-tryptophan production, the glucose conversion rate was decreased because of the reduction of the L-tryptophan production rate and declined production capacity of tryptophan of the strains. Over the entire course of fermentation, the mutants showed lower glucose consumption rate and higher glucose conversion rate than the parental strain. The total glucose conversion rates of TRTHA, TRTHT and TRTHAT were 20.5, 20.1, and 21.2 %, respectively, which were higher than that of the parental strain (19.7 %).

Distribution of metabolic flux

According to the above results, the TRTHAT was the better strain for L-tryptophan production. The metabolic flux distribution of TRTH and TRTHAT during the later fermentation period of L-tryptophan production are presented in Fig. 6. The metabolic flux of tryptophan biosynthesis were changed by the deletion of *ackA* and *tdcD*. Compared with TRTH, the metabolic flux that entered the EMP decreased by 8.37 % and that entered PP was increased by 57.03 % with TRTHAT.

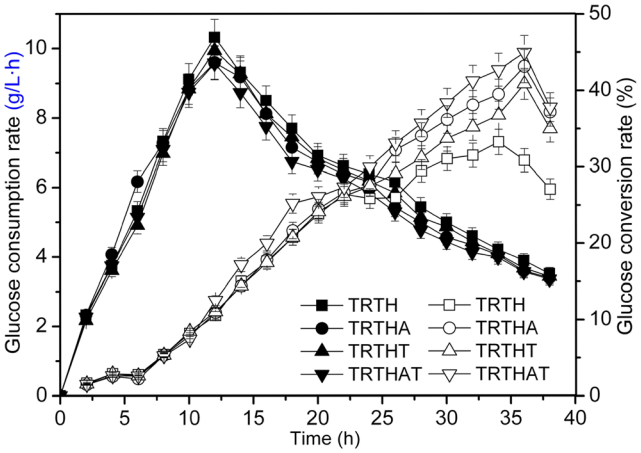

Fig. 5 Effect of gene modification on glucose consumption and conversion rate in L-tryptophan production. *Filled symbols* consumption rate of glucose, *open symbols* conversion rate of glucose ($p<0.05$)

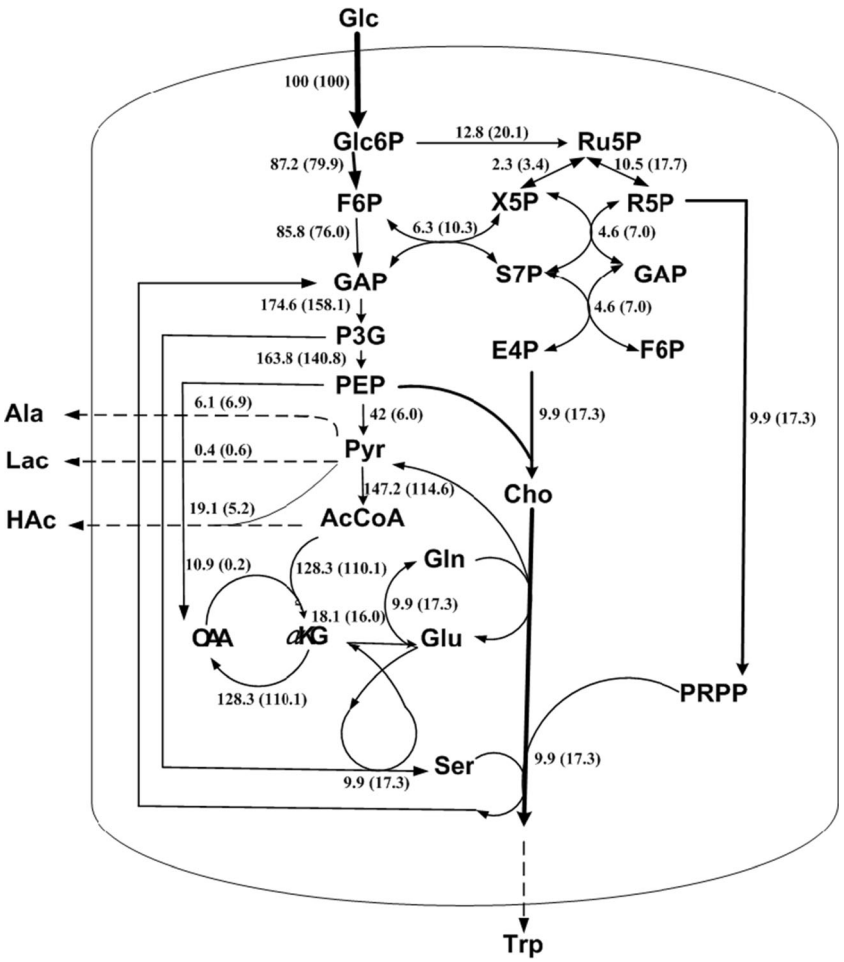

Fig. 6 The metabolic flux distribution of TRTH and TRTHAT during the later fermentation period of L-tryptophan production. *Values in parentheses* metabolic flux of TRTHAT. Metabolites abbreviations: *Glc* Glucose, *GAP* Glyceraldehyde-3-phosphate, *P3G* 3-Phosphoglycerate, *PEP* Phosphoenolpyruvate, *Pyr* Pyruvate, *AcCoA* Acetyl coenzyme A, *Ru5P* Ribulose-5-phosphate, *X5P* Xylulose-5-phosphate, *S7P* Sedoheptulose-7-phosphate, *E4P* Erythrose-4-phosphate, *Cho* Chorismate, *PRPP* 5-Phosphoribosyl pyrophosphate, *OAA* Oxaloacetate, *αKG* α-Ketoglutarate, *Gln* Glutamine, *Glu* Glutamate, *Ala* Alanine, *Lac* Lactate, *Hac* Acetate, *Ser* Serine, *Trp* Tryptophan.

When the *ackA* and *tdcD* were deleted in the TRTH, the flux for acetate decreased to 5.2 and the flux for lactate increased to 0.5. As for TRTHAT, less PEP was used for formation of Pyr, and more PEP and E4P were applied in the biosynthesis of Cho, leading to increasing the metabolic flux of tryptophan formation. The flux of tryptophan formation of TRTHAT was 17.3 %, which was 1.75-times higher than that of TRTH.

Discussion

Acetate metabolism in *E. coli* plays an important role in the control of central metabolism (Wang et al. 2013a). The excretion of acetate results not only in the inefficient utilization of carbon source and the inhibition of cellular growth and protein production but also in the need for cofactor recycling to sustain balanced growth and cellular homeostasis (Chang et al. 1999). Many authors have reported the decreased efficiency of acetate over-producing strains for the high-yield production of recombinant proteins (Eiteman and Altman 2006). The mutants with deletion of *ackA* and/or *tdcD* obtained higher DCW because of the reduction of acetate accumulation, and the strain with lesions in *ackA* and *tdcD* obtained the highest DCW. The PEP/Pyr ratio was decreased because of the presence of a higher concentration of pyruvate, which in turn resulted in a lowered growth rate (Castaño-Cerezo et al. 2009; Wang et al. 2013a). Overall, the maximum specific growth rates of the mutants were lower than that of the parental strain, and that of the *ackA-tdcD* mutant was the lowest. Due to the lower growth rate of the mutants during the early fermentation period, the DCW of the parental strain was higher than that of the mutants during the initial phase of L-tryptophan production. The specific growth rates of the mutations in *ackA* and/or *tdcD* were higher than the parental strain during the later fermentation phase because of the reduction of inhibition caused by the low accumulation of acetate (Wang et al. 2013a).

The inhibition of L-tryptophan formation was decreased by a reduction of the acetate concentration, which led to increasing the production of L-tryptophan (Huang et al. 2011; Cheng et al. 2012; Wang et al. 2013b). The higher concentrations of pyruvate and lactate accumulated during the growth of the mutants could increase the expression level of PEP synthase and the supply of NADPH, which could result in the enhancement of L-tryptophan production because of the improvement of the availability of PEP and the carbon flux through the oxidative branch (Chang et al. 1999; Schmid et al. 2004;

Báez-Viveros et al. 2007). Production of L-tryptophan was improved with the deletion of *ackA* and/or *tdcD* in *E. coli*. In addition, the formation rate of L-tryptophan was increased because of the lower accumulation of acetate (Cheng et al. 2012), and the mutants showed higher production rates of L-tryptophan than the parental strain.

Elimination of the carbon flow toward acetate significantly reduces the acetate yield (De Mey et al. 2007). Deletion of *pta* and/or *ackA* is one direct approach for reducing acetate formation, but this approach is accompanied by an increase in the production of other fermentation products such as pyruvate and lactate (San et al. 2002; De Mey et al. 2007). The excretion of acetate was also decreased using antisense RNAs to partially block the biosynthesis of Pta and AckA (Kim and Cha 2003; Zhou et al. 2011). Compared with the results for the KJ091 strain, the acetate accumulated from the growth of the *tdcDE* deletion mutant was decreased by 50 % (Jantama et al. 2008). As for the L-tryptophan-producing strain, deletion of *ackA* and/or *tdcD* could decrease the accumulation and production rate of acetate because of the reduction of acetate kinase activity, and the mutant with lesions in *ackA* and *tdcD* excreted a lower concentration of acetate than the strains with the single deletion of *ackA* or *tdcD*, which was consistent with the results reported previously (Kumari et al. 1995; Jantama et al. 2008). Acetate was always consumed in the cultures of *E. coli*, and the Pta-AckA and acetyl-CoA synthetase (Acs) pathways are solely responsible for acetate assimilation (Castaño-Cerezo et al. 2009). Overexpression of Acs in *E. coli* results in a significant decrease of acetate accumulation and more efficient acetate assimilation, and the expression of *acs* is induced by acetate (Báez-Viveros et al. 2007; Castaño-Cerezo et al. 2009). In this study, the strains with deletions of *ackA* and/or *tdcD* showed lower consumption rates of acetate than the parental strain because of the lower accumulation of acetate (Wang et al. 2013a).

More pyruvate accumulates in the *E. coli* with disruption of *pta* (Castaño-Cerezo et al. 2009). In the *pta* mutant, lactate production was not the result of the increased expression of lactate dehydrogenase (*ldhA*), but rather from pyruvate accumulation (Zhu and Shimizu 2005). The mutants with deletions of *ackA* and/or *tdcD* accumulated higher concentrations of pyruvate and lactate. The *ackA-tdcD* mutant excreted the highest concentrations of pyruvate and lactate, while the pyruvate and lactate from TRTHT were lower than that of TRTHA (Zhou et al. 2011). Because of the changes in the expression of *ldhA* and *dld* (coding for NAD^+-dependent and NAD^+-independent lactate dehydrogenases, respectively) in the stationary phase, the lactate accumulated in the growth period was recaptured (Vadali et al. 2004; Shi et al. 2005). In the stationary phase of aerobic glucose cultures, the redox environment of the *pta* mutant was more reduced than that of the wild-type strain, which reflects lactate consumption (Chang et al. 1999). The pyruvate and lactate accumulated during the early fermentation period for each strain were reused in the late fermentation period, and pyruvate and lactate were not determined at the end of L-tryptophan fermentation (Shen et al. 2012; Wang et al. 2013a).

Due to the low accumulation of acetate, more glucose was used to synthesize the desired product and the glucose conversion rate was increased (Castaño-Cerezo et al. 2009; Wang et al. 2013a). The DCW and glucose conversion rate were increased because of the reduction of acetate inhibition (Wang et al. 2013a). In the production of L-tryptophan, the glucose conversion rate of the *pta* mutant exhibited a 9.24 % increase as compared with the parental strain (Wang et al. 2013a). From this study, the mutants showed a higher glucose conversion rate than TRTH, and the glucose conversion rate of TRTHAT was highest, 8.16 % higher than the parental strain. The mutants showed lower consumption rates of glucose than the TRTH, and the PEP used for glucose uptake reduced, leading to increasing the production of L-tryptophan (Gosset 2005; Jantama et al. 2008; Shen et al. 2012).

The distributions of metabolic flux were changed with the gene deletion (Huang et al. 2011). In the mutant with lesions in *ackA* and *tdcD*, the metabolic flux was transferred from EMP to PP, and the increase of carbon flux through the PP could increase the supply of E4P, leading to obtaining higher production of L-tryptophan (Shen et al. 2012). The production of NADPH was increased by the enhancement of metabolic flux through PP, which was required by the biosythesis of tryptophan (Báez-Viveros et al. 2007; Wang et al. 2013a). The accumulation of acetate in the culture of TRTHAT was decreased as a result of the smaller metabolic flux through the formation of acetate, meanwhile the lactate excretion was increased with more metabolic flux through the formation of lactate (Huang et al. 2011). Increasing the availability of PEP and E4P is crucial for achieving the maximum flow of carbon into the common pathway for the biosythesis of aromatic amino acids (Wang et al. 2013a). The metabolic flux distribution of TRTHAT indicated less flux of PEP through the formation of Pyr and more flux of PEP and E4P through the biosynthesis pathway of tryptophan, which resulted in a higher production of L-tryptophan obtained in TRTHAT as compared with that of TRTH (Shen et al. 2012).

Though higher production of L-tryptophan obtained in the mutants with the deletion of *ackA* and/or *tdcD*, the L-tryptophan formation of the mutants was limited by the availability of key precursors for tryptophan biosynthesis and the inhibition of acetate (Ikeda 2006; Shen et al. 2012; Wang et al. 2013a). It has been proved that, when *E. coli* grows in minimal medium containing glucose as the carbon source, 50 % of available PEP is consumed by the phosphoenolpyruvate:sugar phosphotransferase system (PTS) for glucose transport (Flores et al. 2002), and the maximum theoretical molar yield from glucose to aromatics synthesis can double in a strain that transports glucose without coupling the process to PEP utilization

(Gosset 2005). With the production of L-phenylalanine by the PTS$^-$ Glc$^+$ strain where glucose was transported and phosphorylated by an alternative transport system, galactose permease (GalP) and glucokinase (Glk), the yield from the glucose in the synthesis of phenylalanine was increased by 57 % when compared to the isogenic PTS$^+$ strains (Báez-Viveros et al. 2007). The excretion of acetate inhibited the formation of the desired product, and the production of L-tryptophan was effectively increased by the reduction of acetate accumulation (Huang et al. 2011; Cheng et al. 2012). The accumulation of acetate was decreased by the disruption of *ackA* and/or *tdcD*, but the formation of L-tryptophan was also inhibited by the acetate accumulated in these mutants. Thus, the production of L-tryptophan can be further improved by increasing the availability of precursors for tryptophan formation and decreasing the accumulation of acetate.

During L-tryptophan production, the accumulation of acetate was decreased by reducing acetate kinase activity through the deletion of *ackA* and/or *tdcD*, which led to the higher production of L-tryptophan and a higher glucose conversion rate. The gene *ackA* was more important for acetate activity than *tdcD* according to the lower concentration of acetate accumulated in the *ackA* mutant than in the strain with the deletion of *tdcD*. The mutants with disruption of the *ackA* and/or *tdcD* genes showed higher accumulations of pyruvate and lactate. The highest L-tryptophan production and glucose conversion rate was obtained in the strain with the lesions in *ackA* and *tdcD*, and the *ackA-tdcD* mutant was the better strain for producing L-tryptophan. The metabolic flux analysis of TRTH and TRTHAT explained why there was a lower accumulation of acetate and a higher production of L-tryptophan obtained in TRTHAT than in the parental strain. Furthermore, how to improve the availability of precursors for tryptophan biosynthesis and reduce the accumulation of acetate for enhancement of L-tryptophan production should be studied further.

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