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ORIGINAL PAPER

Enhanced D-ribose biosynthesis in batch culture of a transketolase-deficient *Bacillus subtilis* strain by citrate

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Abstract In this study, the effects of citrate addition on D-ribose production were investigated in batch culture of a transketolase-deficient strain, *Bacillus subtilis* EC2, in shake flasks and bioreactors. Batch cultures in shake flasks and a 5-l reactor indicated that supplementation with 0.2–0.5 g l^{-1} of citrate enhanced D-ribose production. When *B. subtilis* EC2 was cultivated in a 15-l reactor in a complex medium, the D-ribose concentration was 70.9 g l^{-1} with a ribose yield of 0.497 mol mol^{-1}. When this strain was grown in the same medium supplemented with 0.3 g l^{-1} of citrate, 83.4 g l^{-1} of D-ribose were obtained, and the ribose yield was increased to 0.587 mol mol^{-1}. Addition of citrate reduced the activities of pyruvate kinase and phosphofructokinase, while it increased those of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Metabolic flux distribution in the stationary phase indicated that citrate addition resulted in increased fluxes in the pentose phosphate pathway and TCA cycle, and decreased fluxes in the glycolysis and acetate pathways.

Keywords *Bacillus subtilis* · D-Ribose · Citrate · Transketolase · Metabolic flux

Introduction

D-Ribose, a physiologically and commercially important five-carbon carbohydrate, is not only a constituent component in ribonucleic acids, ATP, NAD, NADP and FAD, but also is used to synthesize riboflavin as well as antiviral and anticancer drugs [33]. D-Ribose can be produced by enzymatic hydrolysis of yeast RNA, chemical synthesis from glucose, arabinose, gluconic acid, xylose and glutamic acid, or microbial fermentation [9]. Hydrolysis of yeast RNA and chemical synthesis suffer from the disadvantages of low yield, high cost and heavy pollution. Microbial fermentation has been proved an effective way for D-ribose production and has gradually replaced the other above-mentioned methods. D-Ribose was first found in a culture of *Penicillium brevicompactum* in 1951 [9]. Since then, people have tried to use various kinds of bacteria to produce D-ribose [9, 32], and it was found that the transketolase (EC 2.2.1.1)-deficient mutants of *Bacillus subtilis* and *B. pumilus* are effective D-ribose producers. Generally, the transketolase-deficient mutants were obtained by genetic engineering or chemical mutation of wild-type strains [8, 28, 29]. Such strains can convert glucose to D-ribose-5-phosphate via the oxidative pentose phosphate pathway. Then, D-ribose-5-phosphate is further dephosphorylated to D-ribose and secreted.

Many attempts have been made to improve D-ribose production, including construction of recombinant strains, selection of suitable carbon and nitrogen sources, and so on [26]. Various carbon sources (D-glucose, D-fructose, D-mannose, D-sorbitol, D-mannitol, sucrose, molasses, starch, etc.) and nitrogen sources (dried yeast, yeast extract, peptone, corn steep liquor, fishmeal, casein hydrolysate, amino acid mixture, some inorganic nitrogen, etc.) have been used as nutrients. Glucose and corn steep liquor are particularly effective for production of D-ribose [9, 32]. To increase D-ribose production, some substrates metabolized only via the pentose phosphate pathway, such as D-gluconic acid, D-xylose, D-xylitol, and D-arabinose, were supplied together with glucose [7, 10, 28, 29]. For example, 50 g l^{-1}

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of D-gluconic acid were supplied together with 100 g l^{-1} of D-glucose to obtain 45 g l^{-1} of D-ribose in 84 h [10], and the yield of D-ribose was increased from 0.24 to 0.37 mol mol^{-1} based on total carbon sources consumed. A mixture of D-glucose and D-xylose was used to increase the D-ribose concentration and productivity in fed-batch fermentation [28, 29]. D-Ribose production is also dependent on the nitrogen and phosphorus substrates. For instance, addition of aromatic amino acids to corn steep liquor improved the yield of D-ribose and inhibited the formation of by-products [22]. Recently, it was observed that a high phosphorus or ammonium sulfate concentration led to lower D-ribose yield [34]. Although various strategies have been used to enhance D-ribose concentration, the overall D-ribose yield based on carbon source still remains not high.

It is known that some metabolites can alter metabolic flux via the cellular regulatory mechanism; consequently, metabolic flux can be redirected to desirable pathways. Citrate regulates glycolysis by control of the activities of phosphofructose kinase (EC 2.7.1.11, PFK) and pyruvate kinase (EC 2.7.1.40, PYK). In *B. subtilis*, co-metabolism of citrate with glucose leads to zero acid production over a wide range of growth rates by reducing glycolytic flux drastically [15, 16]. Citric acid addition has been observed to increase inosine production by 18% [5]. Unlike *Escherichia coli*, *B. subtilis* transports citric acid readily under aerobic conditions. The citrate transporters, CitH and CitM, in *B. subtilis* carry citrate–metal ion complex other than free citrate. They are specific for metal ion. CitM transports the complex of citrate with Mg^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+} and Zn^{2+}, while CitH transports the complex of citrate with Ca^{2+}, Sr^{2+} and Ba^{2+} [23, 25]. In this study, we found that D-ribose production and yield were enhanced by supplementing with citric acid to limit the formation of by-products in batch culture of a transketolase-deficient strain, *B. subtilis* EC2.

Materials and methods

Strain

A transketolase-deficient strain, *B. subtilis* EC2, was used in the present studies. This strain was stored in 25% (v/v) glycerol at −20°C.

Media

The slant medium consisted of (per liter): sorbitol 5 g, tryptone (Sinopharm Chemical Reagent Co., Shanghai, China) 10 g, NaCl 2 g, yeast extract paste (Sinopharm Chemical Reagent Co.) 2 g, KH_2PO_4 1 g, K_2HPO_4 2 g and agar 22 g. The medium for inoculum culture was composed of (per liter): D-glucose·H_2O 20 g, yeast extract paste 3 g, tryptone 10 g, KH_2PO_4 1 g and K_2HPO_4 3 g. The pH was adjusted to 7.0. The fermentation medium contained (per liter): D-glucose·H_2O 180 g, corn steep liquor (CSL, Lukang Pharmaceutical Group Co., Shandong, China) 27 g, $(NH_4)_2SO_4$ 9 g, $CaCO_3$ 20 g, $MnSO_4·H_2O$ 0.05 g and $MgSO_4$ 0.05 g. All media were sterilized at 121°C for 30 min.

Culture conditions

The strain was activated by inoculation on the nutrient slant and incubation for 40 h at 37°C. The primary inoculum culture was prepared by transferring a loop of cells from the slant to 20 ml inoculation medium in a 250-ml flask, and the cells were incubated for 47 h at 37°C and 220 rpm. The secondary inoculum culture was obtained by transferring 7.5 ml of the primary inoculum culture to 500-ml flasks containing 67.5 ml inoculation medium, and incubating at 37°C and 220 rpm for 9 h. For the experiments carried out in shake flasks, the secondary inoculum culture was inoculated to 250-ml flasks each containing 22.5 ml of the fermentation medium at an inoculum size of 10% (v/v), then the flasks were incubated at 220 rpm and 37°C. All the shake flask experiments were performed at least in triplicate. For the experiments carried out in the fermentor, the secondary inoculum cultures were combined to inoculate 2.5 or 10 l of the fermentation medium in a 5-l reactor (Model RIBE-5, ECUST, Shanghai, China) or a 15-l reactor (FUS-15L, Guoqiang Co., Shanghai, China) with an inoculum size of 6% (v/v). The culture temperature was 37°C, and the pH was 6.0 automatically adjusted with 3 M NaOH or 2 M H_2SO_4. Dissolved oxygen was maintained above 30% by adjusting the agitation speed. The initial aeration and agitation rates in the 5-l reactor were 1.6 vvm and 600 rpm, while those in the 15-l reactor were 1 vvm and 400 rpm, respectively. The O_2 and CO_2 concentrations in the exhaust gas stream from the 15-l reactor were continuously measured by a gas analyzer (PS6000, Hartmann Co., Chongqing, China), and the aeration rate was measured with a mass flow meter. All the on-line data were collected and stored in the PC. All experiments in the fermentors were carried out twofold or in triplicate.

Analytical methods

The cell concentration was analyzed by measuring the optical density at 650 nm (OD_{650}) using a spectrophotometer. The culture sample was diluted with 0.3 M HCl to dissolve the $CaCO_3$ in the medium. The optical density was converted to dry cell weight (DCW, in g l^{-1}) according to a linear relationship between the OD_{650} and DCW. One

unit of OD_{650} was equivalent to 0.367 g l^{-1} (DCW). The concentration of D-glucose was estimated with an enzymatic assay kit containing glucose oxidase (Kexin Biotech, Shanghai, China). The concentration of D-ribose was determined by the orcinol method, which was based on the dehydration of D-ribose to produce furfural in HCl medium, and the reaction of furfural with 3,5-dihydroxytoluene and Cu^{2+} to form a color complex [31]. The concentrations of acetic acid, citric acid and gluconic acid were quantified by an ion chromatography system (ICS-1500; Dionex, Sunnyvale, CA) equipped with a 4 × 250-mm anion-exchange column (AS11-HC), a heated conductivity detector (DS6) and an anion self-regenerating suppressor (ASRS ULTRAII; 4 mm). The temperatures of the column and heated conductivity detector were 30°C. The mobile phase was 1.0 mM KOH at a flow rate of 1.0 ml min^{-1}.

All enzymatic activities were measured by monitoring the change of NADPH or NADH. The activity of 6-phosphogluconate dehydrogenase (EC 1.1.1.44, 6PGDH) was determined by the oxidation of 6-phosphogluconate [24]. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6PDH) was estimated as described by Bergmeyer [2]. PFK was assayed by the method of Wood [36]. PYK was measured by the method of Peng and Shimizu [30]. One unit of activity was defined as the amount of enzyme needed to form 1 mol of NADPH or to consume 1 mol of NADH per minute. The total protein concentration in crude cell extract was measured by Bradford's method [4].

Metabolic flux analysis (MFA)

The metabolic flux distribution in the stationary phase was analyzed. Analysis of the fluxes in the stationary phase was to avoid calculation of the fluxes related to cell growth on the complex nitrogen source in the growth phase. In the stationary phase, the cells stopped growing, and product formation was only related to glucose consumption. In this study, the glycolytic pathway, TCA cycle, pentose phosphate pathway and acetate pathway were considered for MFA, as shown in Fig. 5. The Entner Doudoroff pathway, glyoxylate shunt and phosphoenolpyruvate (PEP) carboxylase were not considered here [6]. The PEP carboxykinase is repressed by glucose and was therefore not considered [11]. Under aerobic conditions, acetate is synthesized through the pathways of *poxB* and *ackA-pta* with glucose as the substrate. It is reported that the *ackA-pta* pathway dominates in the exponential phase, and the *poxB* pathway dominates in the stationary phase [12], so the *ackA-pta* pathway was not included in our study.

Based on the pseudo-steady state assumption, the general stoichiometric equations for the cellular metabolic network can be given as follows:

$$\boldsymbol{Sv} = \boldsymbol{b}$$

where $\boldsymbol{S}$, $\boldsymbol{v}$ and $\boldsymbol{b}$ indicate the stoichiometric coefficient matrix of the metabolic network, the vector of reaction fluxes and the vector of the metabolite accumulation rates, respectively.

Results

Effect of citric acid on D-ribose production in shake flasks and a 5-l reactor

D-Ribose is a metabolite produced in the pentose phosphate pathway. To improve the yield of D-ribose, it is important to limit the carbon flux in the glycolysis pathway and to enhance the flux in the oxidative pentose phosphate pathway. Some studies indicate that citrate can suppress the flux of glycolytic pathway by regulation of enzymes levels [5, 15, 16]. To study the effects of citric acid on growth of *B. subtilis* and D-ribose production, shake flask cultures where citrate was added at different concentrations were carried out. The experiments revealed that when supplemented with 0.2–0.55 g l^{-1} of citric acid, the D-ribose production and yield were enhanced compared with the control, as shown in Table 1. In the presence of 0.3 g l^{-1} of citric acid, the concentration and yield of D-ribose were improved by 10.7 ± 0.5 and 10.8 ± 0.6%, respectively, as compared with that without citrate addition. Significance analysis was performed, and the result of the *P* value of 0.003, which was less than 0.05, indicated that the increases were statistically significant.

Table 1 Effect of citrate concentration on D-ribose production in shake flask culture

Citrate (g l^{-1})	DCW (g l^{-1})	Glucose consumed (g l^{-1})	D-ribose (g l^{-1})	Yield (mol mol^{-1})
0	4.93 ± 0.04	148.47 ± 1.05	41.13 ± 0.66	0.333 ± 0.007
0.10	4.85 ± 0.04	148.29 ± 1.14	41.49 ± 0.42	0.336 ± 0.001
0.20	4.96 ± 0.06	148.40 ± 1.57	43.71 ± 0.26	0.354 ± 0.004
0.30	4.77 ± 0.08	148.32 ± 0.71	45.54 ± 0.32	0.369 ± 0.002
0.55	4.91 ± 0.06	145.01 ± 0.94	42.40 ± 0.46	0.351 ± 0.002
0.70	5.12 ± 0.04	141.27 ± 1.61	38.90 ± 0.11	0.331 ± 0.003

To further investigate the culture patterns of the transketolase-deficient *B. subtilis* EC2, batch cultures were carried out in a 5-l bioreactor. According the above shake flask experiments, the citric acid concentrations of 0.2, 0.3 and 0.5 g l^{-1} were examined. Figure 1a depicts the profiles of cell growth, glucose consumption and D-ribose production in the medium without supplementation of citrate. A maximum cell density (DCW) of 11.2 g l^{-1} and a maximum specific growth rate of 0.325 h^{-1} were obtained. The final D-ribose concentration was 71.7 g l^{-1} with a ribose yield of 0.503 mol mol^{-1}. The higher ribose production in the reactor could be attributed to the controlled pH and DO, which could not be controlled in the flasks. Figure 1b shows the trends when 0.2 g l^{-1} of citrate was supplemented. Cell growth was little affected: the cell density achieved 11.04 g l^{-1}, and the maximum specific growth rate was 0.317 h^{-1}. The final D-ribose concentration was 77.1 g l^{-1} with a yield of 0.547 mol mol^{-1}. When *B. subtilis* EC2 was cultivated in the medium supplemented with 0.3 g l^{-1} of citrate, the cell density reached 11.0 g l^{-1}, and the D-ribose concentration was 82.3 g l^{-1} with a yield of 0.578 mol mol^{-1} as shown in Fig. 1c. The specific growth rate in the exponential growth phase was 0.319 h^{-1}. However, when 0.5 g l^{-1} of citrate was added, the cell density and D-ribose concentration decreased to 10.9 g l^{-1} and 75.0 g l^{-1}, respectively (Fig. 1d). In addition, D-glucose was not used completely: only 93.3% of glucose was consumed, even though the culture time was extended to 42 h. These experiments also revealed that citric acid at 0.2–0.5 g l^{-1} could enhance D-ribose production, especially at 0.3 g l^{-1}, consistent with those carried out in shake flasks. In addition, the glucose consumption rate also decreased with increasing citrate concentration.

Batch fermentation in a 15-l reactor

To understand the effect of citrate on D-ribose fermentation in detail, batch culture was carried out in a 15-l fermentor, where the O_2 and CO_2 contents in the exhaust gas stream could be followed. Figure 2a depicts the profiles of cell growth, glucose consumption and D-ribose production in a typical batch culture without citrate supply. The cells grew at a specific growth rate of 0.314 h^{-1} in the exponential growth phase. A maximum dry cell weight of 11.3 g l^{-1} was obtained. D-Ribose was produced after the late-exponential growth phase, and a maximum concentration of 70.9 g l^{-1} was obtained with a D-ribose yield of 0.497 mol mol^{-1}. The major by-products were acetate and gluconate, which were formed simultaneously with D-ribose and reached 12.3 and 27.5 g l^{-1}, respectively. With the cell growth, q_{CO_2} and q_{O_2} increased correspondingly. At 4.5 h or so, q_{CO_2} and q_{O_2} began to decrease rapidly, but the peak of

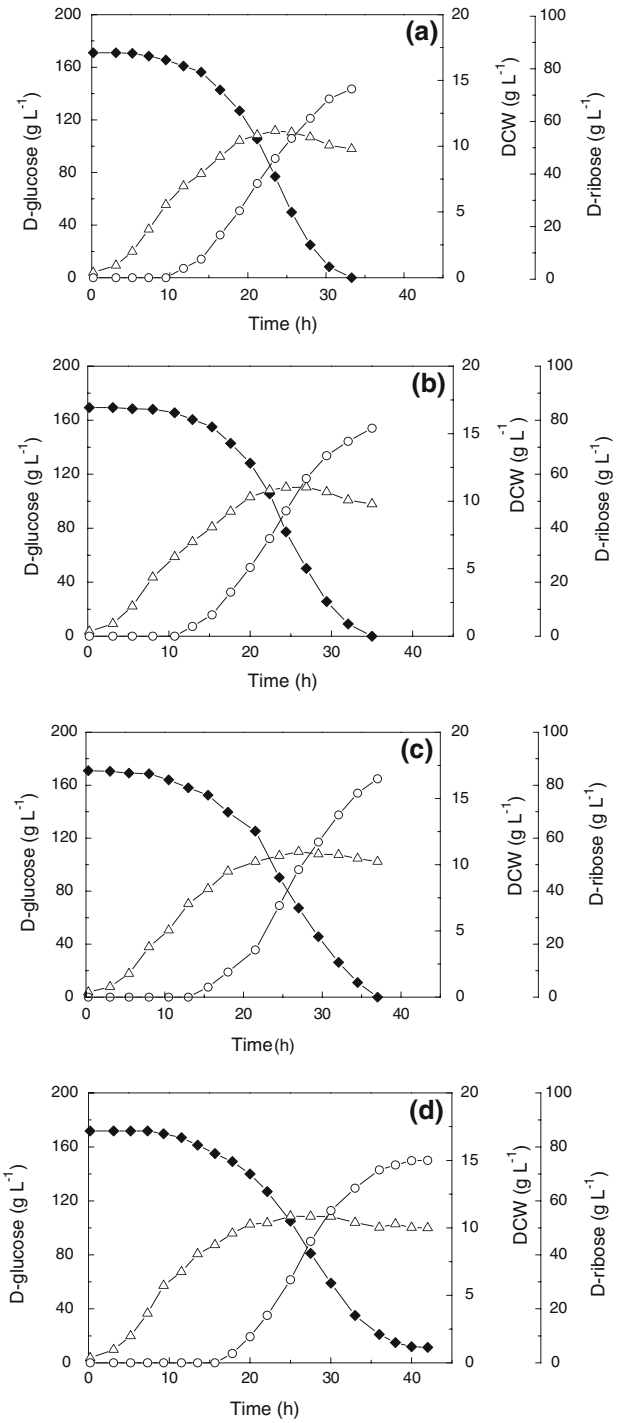

Fig. 1 Profiles of glucose (*filled diamond*), DCW (*triangle*) and D-ribose (*circle*) in batch culture of *Bacillus subtilis* strain EC2 grown in medium supplemented with different concentration of citrate: **a** 0 g l^{-1}, **b** 0.2 g l^{-1}, **c** 0.3 g l^{-1}, **d** 0.5 g l^{-1}

q_{O_2} appeared later. Then q_{CO_2} and q_{O_2} maintained relatively constant values until the start of the stationary phase. During the time interval of 0–6 h, the respiration quotient (RQ) was above 1, which was thought to be due to utilization of some kinds of organic acids in CSL together with

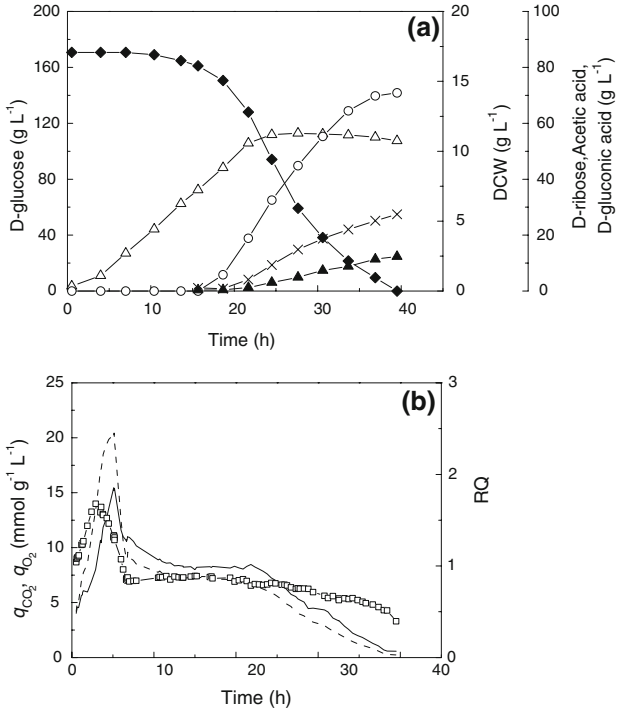

Fig. 2 Batch culture of *Bacillus subtilis* strain EC2 in a 15-l reactor without citrate addition. **a** Profiles of cell growth, glucose consumption and product formation; **b** profiles of q_{O_2}, q_{CO_2} and RQ. (*Filled diamond*) glucose, (*triangle*) DCW, (*circle*) D-ribose, (*multiplication symbol*) D-gluconic acid, (*filled triangle*) acetic acid, (*square*) RQ, (*dotted line*) q_{CO_2}, (*solid line*) q_{O_2}

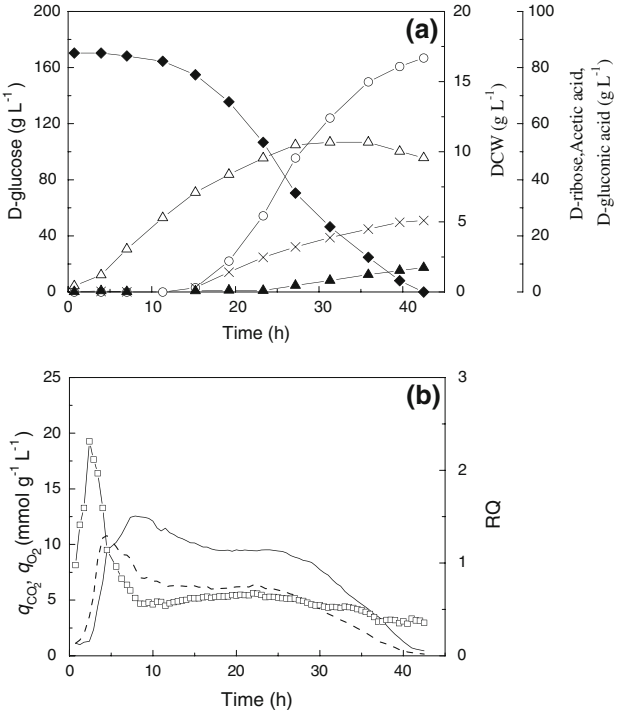

Fig. 3 Batch culture of *Bacillus subtilis* strain EC2 in a 15-l reactor with 0.3 g l^{-1} of citrate. **a** Profiles of cell growth, glucose consumption and product formation; **b** profiles of q_{O_2}, q_{CO_2} and RQ. (*Filled diamond*) glucose, (*triangle*) DCW, (*circle*) D-ribose, (*multiplication symbol*) D-gluconic acid, (*filled triangle*) acetic acid, (*square*) RQ, (*dotted line*) q_{CO_2}, (*solid line*) q_{O_2}

glucose. Then RQ decreased gradually (Fig. 2b) until the organic acids (such as lactic acid, not shown) were exhausted. During the period between 27 and 36 h, the RQ was maintained between 0.62–0.75, and the highest productivity of D-ribose (2.7 g l^{-1} h^{-1}) was obtained during the late growth and initial stationary phases.

Batch cultures performed in the 5-l reactor indicated that supplementation with 0.3 g l^{-1} of citric acid could increase the concentration of D-ribose most effectively. Figure 3a presents the patterns of cell growth, glucose consumption, D-ribose and by-products production with the addition of 0.3 g l^{-1} citric acid in the 15-l reactor. The maximum DCW was 10.6 g l^{-1}, slightly lower than that without citrate addition (Fig. 2a). A significantly increased D-ribose production (83.4 g L^{-1}) was obtained. The yield of D-ribose based on consumed glucose was 0.587 mol mol^{-1}. Glucose was consumed completely, similar to the case without citrate addition, but the rate of glucose consumption was lowered to some extent. Furthermore, by-product formation was restricted: 8.69 g l^{-1} acetic acid and 25.5 g l^{-1} gluconic acid were produced at the end of culture. Although the effect of citrate was significant on acetate production, its effect on gluconate was insignificant based on the significance test. The trends of q_{CO_2}, q_{O_2} and RQ (Fig. 3b) were similar to those without citrate, but the q_{CO_2} and q_{O_2} declined more slowly in the growth phase and late stationary phase. After 24 h, the q_{CO_2} was higher than that without citrate addition and could be related with higher D-ribose production during this period. The q_{O_2} after 7 h was obviously higher than that without citrate addition and resulted in lower RQ. Replicative experiments indicated that the RSDs for gluconic acid, D-ribose and acetic acid were 2.9, 3.2 and 6.5% for the control and 4.5, 2.78 and 5.6% for the experiments supplied with citrate in the 15-l reactor.

Effect of citrate on the activities of enzymes

To understand the mechanism of citrate in fermentation, the activities of some key enzymes were measured. The activities of PFK, PYK, G6PDH, and 6PGDH in the cells cultured in the 15-l reactor supplemented with and without citrate are compared in Fig. 4. When 0.3 g l^{-1} citrate was present, the specific activities of PFK and PYK were decreased by 33.2 and 38.6%, respectively, suggesting that the glycolysis pathway was suppressed due to the citrate supplementation. Unlike PFK and PYK, the specific activities of G6PDH and 6PGDH were higher in the case of citrate addition. They were increased by 15.4 and 15.2%, respectively.

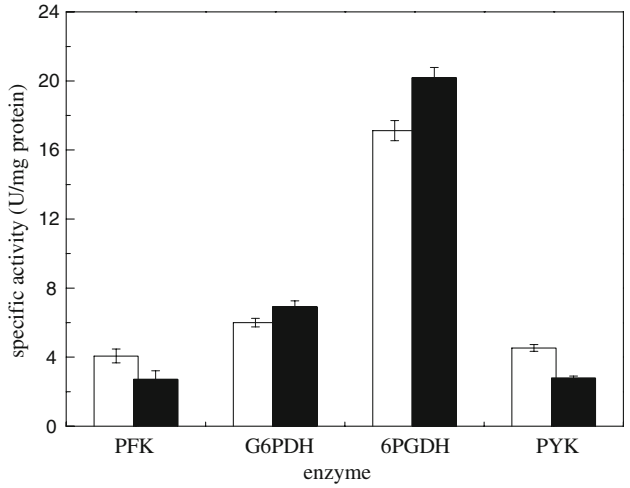

Fig. 4 Specific activities of enzymes in the cells cultured in the 15-l reactor during the stationary phase. *White column* without citrate, *black column* with citrate

Discussion

In this study, it was found that citric acid had positive effects on both the final concentration and yield of D-ribose. In the fermentation carried out in the 15-l reactor, the presence of 0.3 g l^{-1} of citrate resulted in increased final concentration and yield of D-ribose by 17.6 and 18.1%, respectively. Furthermore, formation of by-products was also affected by citrate addition. The D-gluconic acid concentration reached 27.5 g l^{-1} in the culture without the addition of citrate, while it decreased to 25.5 g l^{-1} with citrate supplementation. Addition of citrate resulted in 8.69 g l^{-1} of acetic acid, which was 70.7% of that without citrate supplementation. Some metabolic properties in the stationary phase are summarized in Table 2. It can be seen that the average specific D-ribose production rate was improved, and the average specific production rates of both acetic acid and gluconic acid were decreased when citrate was added. Although the whole fermentation period in the presence of citrate was prolonged, the D-ribose productivity was still enhanced. CO_2 evolution in the absence of citrate was lower than that in the presence of citrate after 24 h due to lower flux through the oxidative pentose phosphate pathway and TCA cycle to release less CO_2. The metabolic flux distribution in the stationary phase was also analyzed for the cultures supplied with and without citrate (Fig. 5). The carbon recovery was between 94.0 and 97.8% in the stationary phase. It can be seen from Fig. 5 that the fluxes through the glycolysis pathway (G6P to PYR) and acetate pathway (PYR to acetate) were attenuated owing to citrate supplementation. As a result, the flux through the TCA cycle greatly enhanced and RQ decreased even though the flux through the pentose phosphate pathway was increased. The significant increase in the flux to the TCA cycle also contributed to enhanced q_{CO_2}. Therefore, citrate addition promoted more carbon flux to the pentose phosphate pathway and restricted the flux to EMP and acetate pathways.

Generally, citrate is regarded as an inhibitor of PFK and enhances the inhibitive effect of ATP. The PFK from some eukaryotic cells is inhibited by citrate, but the regulatory features of PFK in *B. subtilis* still remain unclear [17, 21, 27]. The activity of PFK of many bacteria, such as *E. coli* and *B. stearothermophilus*, is not affected by citrate, but is inhibited by phosphoenolpyruvate (PEP) [1, 3, 13, 14]. In addition, as the first metabolite of the TCA cycle, high citrate concentration is a signal of excessive precursors of the cell component, leading to depression of the TCA cycle. However, in the present study, citrate addition did not decrease, but increased the flux through the TCA cycle. Some studies indicate that glutamine and intermediates of the TCA cycle added to the culture medium do not reduce the flux through EMP during the course of *B. subtilis* growth. The effect of citrate is due to inhibition of glycolysis rather than serving as a supplement of TCA cycle metabolites [15]. Citrate is readily transported by *B. subtilis,* and the transport is coupled with uptake of divalent metal ions, such as Ca^{2+} and Mg^{2+} [23, 25]. It has been shown that Ca^{2+} can inhibit PYK activity, and the *B. subtilis* PYK activity decreases with increasing Ca^{2+} concentration [16, 20]. Therefore, addition of citrate decreased PYK activity indirectly, and in turn, elevated intracellular PEP pool and reduced intracellular pyruvate concentration. As a result, reduced pyruvate decreased acetate formation, but affected gluconate production less. Goel et al. found that co-metabolism of glucose and citrate eliminated acetate production [15, 16], while in the present study acetate was still formed, but was decreased by 29.3%. The discrepancy may be due to the complex medium used in the

Table 2 Effects of citrate on metabolic properties in the stationary phase (27–36 h) of batch culture in the 15-l reactor

Citrate (g l^{-1})	Average specific rate (mmol g^{-1} h^{-1})						RQ	Productivity[a] (g l^{-1} h^{-1})
	Glucose	Ribose	Acetate	Gluconate	CO_2	O_2		
0	2.64	1.60	1.03	0.505	2.76	3.22	0.62–0.75	1.79
0.3	2.75	1.95	0.694	0.352	3.60	6.46	0.54–0.66	1.96

[a] Average for the whole fermentation process

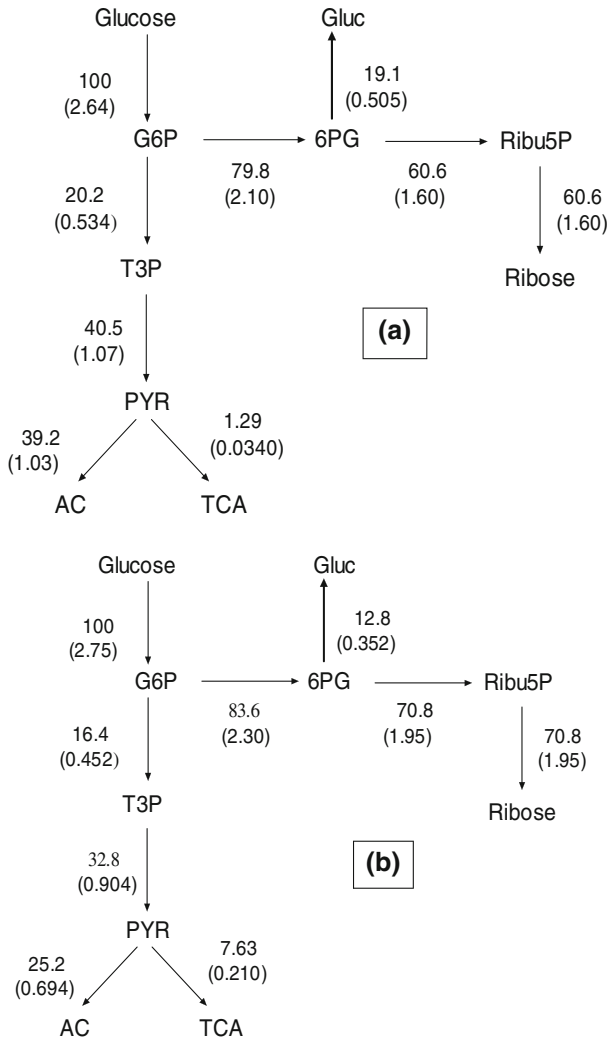

Fig. 5 Metabolic flux distribution in the stationary phase in batch culture of *Bacillus subtilis* strain EC2 in the 15-l reactor. The figures are normalized flux values, while those in *parentheses* are real fluxes (mmol g^{-1} DCW h^{-1}) **a** in the absence of citrate, **b** in the presence of citrate

present study, because some studies indicate that a complex nitrogen source can enhance acetate production [18, 35]. When yeast extract is added, acetate production is increased by 12-fold compared with that in a chemically defined medium [35]. A complex nitrogen source usually contains abundant free amino acids, and certain amino acids can be degraded to acetyl-CoA, which can be further converted to acetate [19]. PEP is an inhibitor of PFK in most bacteria [21]. Thus, reducing activities of PFK and PYK lead to decreased flux of glycolysis. The decline of glycolysis flux causes the pentose phosphate pathway flux to increase, and accordingly enhances D-ribose production.

In summary, citrate addition was effective for D-ribose production. When 0.3 g l^{-1} citrate was supplemented in the medium, the D-ribose yield and production were increased by 18.1 and 17.6% respectively, in the 15-l bioreactor. Addition of citric acid redistributed metabolic flux to D-ribose formation, and suppressed formation of acetate and gluconate. Although many methods to increase D-ribose production have been reported, the yield of D-ribose based on glucose is rather low. In this study, the supply of citrate was adopted, and the molar yield of D-ribose reached 0.587 mol mol^{-1}.

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