Metabolic Engineering 33 (2016) 12–18

Contents lists available at ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/ymben

Metabolic engineering of *Corynebacterium glutamicum* for the de novo production of ethylene glycol from glucose

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ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form
14 September 2015
Accepted 30 October 2015
Available online 7 November 2015

Keywords:
Ethylene glycol
Serine
Metabolic engineering
Pathway optimization

ABSTRACT

Development of sustainable biological process for the production of bulk chemicals from renewable feedstock is an important goal of white biotechnology. Ethylene glycol (EG) is a large-volume commodity chemical with an annual production of over 20 million tons, and it is currently produced exclusively by petrochemical route. Herein, we report a novel biosynthetic route to produce EG from glucose by the extension of serine synthesis pathway of *Corynebacterium glutamicum*. The EG synthesis is achieved by the reduction of glycoaldehyde derived from serine. The transformation of serine to glycoaldehyde is catalyzed either by the sequential enzymatic deamination and decarboxylation or by the enzymatic decarboxylation and oxidation. We screened the corresponding enzymes and optimized the production strain by combinatorial optimization and metabolic engineering. The best engineered *C. glutamicum* strain is able to accumulate 3.5 g/L of EG with the yield of 0.25 mol/mol glucose in batch cultivation. This study lays the basis for developing an efficient biological process for EG production.

1. Introduction

With the growing concern over environmental crisis and fossil-energy depletion, development of sustainable biological process using renewable bioresources to produce energy, materials and chemicals is becoming an appealing approach for chemical industry. The fast development of analytical and engineering tools from metabolic engineering, systems and synthetic biology in the past two decades has substantially accelerated the process of strain engineering to produce both natural and non-natural products (Chen et al., 2010, 2015; Chen and Zeng, 2013). Biological production of bulk chemicals at commercial scale, such as butanol (Lan and Liao, 2013), 1,3-propanediol (Nakamura and Whited, 2003) and 1,4-butanediol (Yim et al., 2011), has been achieved by intensive strain modifications integrating different rational engineering tools. Herein, we report the production of ethylene glycol (EG), another important large-volume commodity chemical, from renewable feedstock (e.g. glucose) by de novo pathway design and metabolic engineering.

EG is a very important platform chemical which can be used as raw material for polymers, anti-freezing agent and coolant etc. The primary application of EG is to synthesize over 25 million tons of polyethylene terephthalate (PET) annually. Currently, EG is mainly produced based on chemical route using ethylene derived from the petrochemical industry. Although ethylene could now be produced by chemical dehydration of bioethanol (Zhang and Yu, 2013), a more economical route to manufacture EG directly from bio-feedstock is highly desirable. Development of low-cost fermentation process to produce EG from carbohydrate is an important alternative towards such goal. To the best of our knowledge, no natural microorganism can produce EG from glucose. Only marginal accumulation of EG as a metabolic byproduct was previously identified during the degradation of arabinose by some microorganism such as *Caldicellulosiruptor saccharolyticus* (Isern et al., 2013). Recently, the biosynthesis routes of EG from pentoses have been clarified and metabolic engineering of *Esherichia coli* has enabled the direct fermentation of arabinose or xylose to produce EG (Liu et al., 2013; Stephanopoulos et al., 2013). However, the theoretical yield of the proposed pathways is only 1 mol EG/mol pentose (0.4 C-mol/C-mol), making this approach economically unfavorable. Furthermore, other abundant sugars, such as sucrose and glucose, cannot be utilized as substrates based on the proposed pentose degradation pathway. The need for a more efficient process led us to evaluate other potential pathways for EG production.

In this study, we proposed that EG production can be achieved by the extension of serine synthesis pathway as illustrated in Fig. 1A (see detailed description in the Results session). Serine is a natural amino acid that can be synthesized by most of microorganisms. High accumulation of serine has been previously demonstrated by metabolic engineering of *Corynebacterium glutamicum* or *E. coli* (Gu

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http://dx.doi.org/10.1016/j.ymben.2015.10.013
1096-7176/

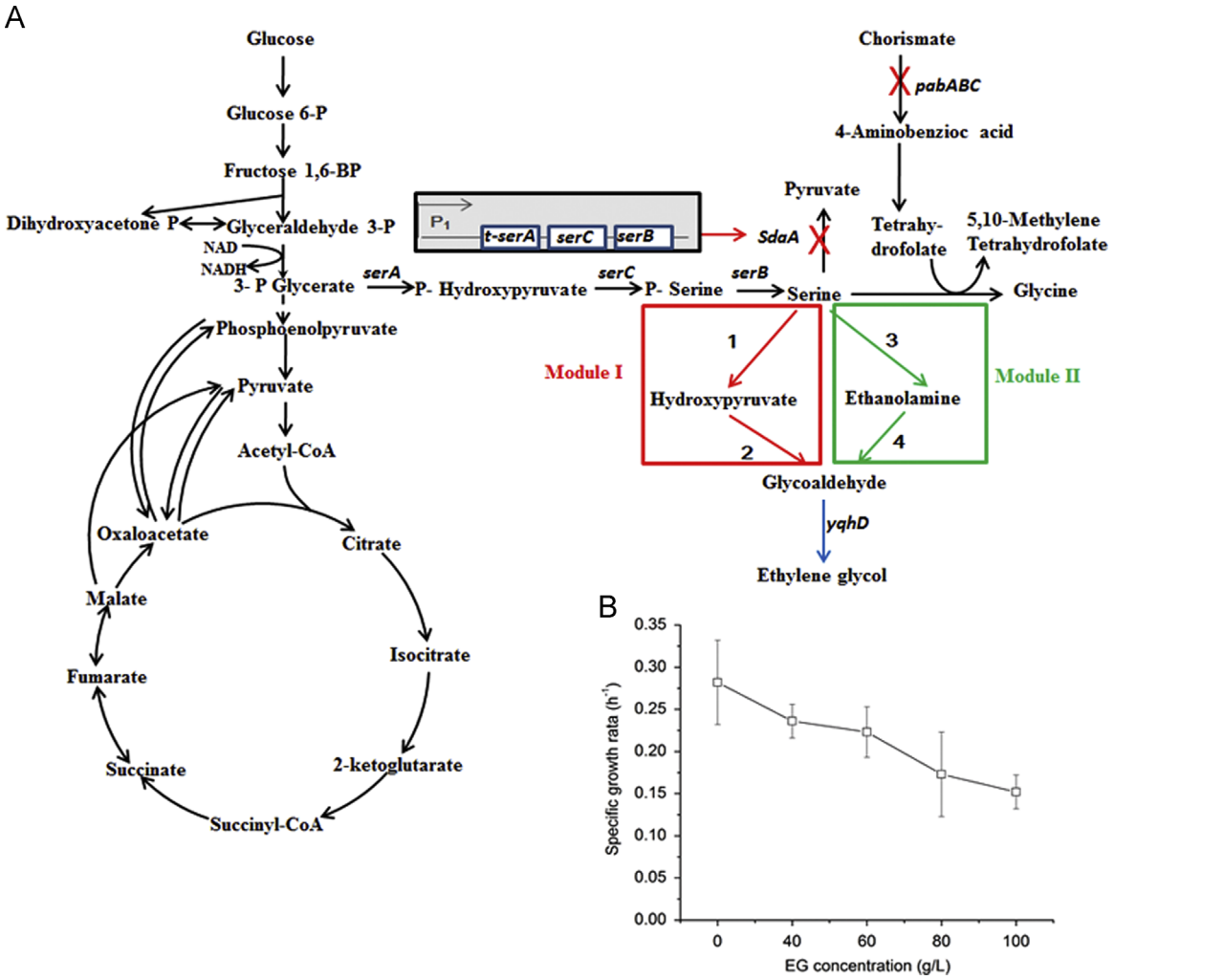

Fig. 1. Proposed routes for ethylene glycol (EG) production derived from serine synthesis pathway (A) and effect of EG for the cell growth of *Corynebacterium glutamicum* (B). (A) Serine can be transferred into glycoaldehyde via two synthetic modules: (1) deamination of serine to hydroxypyruvate by aminotransaminase or amino acid dehydrogenase, and decarboxylation of hydroxypyruvate to glycoaldehyde by α-ketoacid decarboxylase (module I); (2) decarboxylation of serine to ethanolamine by serine decarboxylase, and oxidation of ethanolamine to glycoaldehyde by monoamine oxidase (module II). Glycoaldehyde can be reduced to EG by alcohol dehydrogenase such as *yqhD*. To enhance the precursor supply for EG synthesis, the *pabABC* operon encoding the aminodeoxychorismate synthase and aminodeoxychorismate lyase was deleted. *SdaA* gene encoding serine deaminase was substituted by an artificial *serACB* operon, containing truncated *serA* (with a deletion of 197 amino acids at the C terminus), *serC* and *serB* under the control of a strong constitutive promoter. (B) To evaluate the toxicity of EG, *C. glutamicum* was cultured in LB medium with 25 g/L glucose and different concentrations of EG at 30 °C and 200 rpm.

et al., 2014; Stolz et al., 2007; Zhu et al., 2014). *C. glutamicum* is a gram-positive bacterium which can utilize various substrates for the production of different amino acids in industry (Bommareddy et al., 2014; Chen, et al., 2014; Hasegawa et al., 2013). Recently, *C. glutamicum* has also been engineered to produce other bulk chemicals such as isobutanol, cadaverine, succinate, etc. (Blombach et al., 2011; Buschke et al., 2011; Litsanov et al., 2012). In this study, we selected a prophage-free *C. glutamicum* strain MB001 (Baumgart et al., 2013) as the chassis to reconstruct the EG synthesis route based on the extension of its natural serine synthesis pathway. By systematical enzyme screening and combinatorial pathway assembling, we demonstrated that it was possible to directly produce EG from glucose by metabolically engineered *C. glutamicum*.

2. Materials and methods

2.1. Bacterial strains and plasmids

Strains and plasmids used in this study are listed in Table 1. *E. coli* DH5αMCR (Invitrogen) was used for the construction of pK18mob*sacB* derived suicide vector for gene deletion and substitution in *C. glutamicum* (Schäfer et al., 1994). *E. coli* DH5α was routinely used for the construction of other plasmids. *C. glutamicum* MB001 is a prophage-free strain derived from wildtype *C. glutamicum* ATCC13032 (Baumgart et al., 2013). Gibson assembly cloning kit (NEB) was used to construct all of the plasmids (Gibson et al., 2009).

2.2. Construction of suicide vectors and C. glutamicum mutants

Gene knockout or substitution in *C. glutamicum* MB001 was achieved by a two-step homologous recombination process based on the suicide vector pK18mob*sacB* as described before (Chen et al., 2011). All of the constructed strains were verified by colony PCR using appropriate primers.

To delete *pabABC* operon encoding the aminodeoxychorismate synthase and aminodeoxychorismate lyase (nucleotides 1,054,917–1,057,051), pK18mob*sacB-pabABC* was constructed. Primers pabABC-up-F and pabABC-up-R were used to amplify a 1000-bp fragment of the upstream of *pabABC* and primers pabABC-down-F and pabABC-down-R was used to amplify a 1000-bp fragment of the downstream