Biochemical Engineering Journal 129 (2018) 113–118

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Short communication

Metabolic engineering of *Escherichia coli* for the production of glyoxylate from xylose

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ARTICLE INFO

Article history:
Received 1 September 2017
Received in revised form 27 October 2017
Accepted 3 November 2017
Available online 10 November 2017

Keywords:
Glyoxylate
Glycolate oxidase
Catalase
Metabolic engineering
Xylose
Escherichia coli

ABSTRACT

Glyoxylate is an important chemical building block which is currently produced by chemical or electrochemical oxidation methods We herein described the achievement of microbial glyoxylate production from renewable biomass by metabolic engineering of *Escherichia coli*. The synthetic ribulose-1-phosphate pathway was expressed to produce glycolate from xylose. Additional genomic modifications including the inactivation of malate synthase genes *aceB*/*glcB* and glyoxylate carboligase gene *gcl* were performed to prevent the consumption of glyoxylate. The constructed strain was found to accumulate 0.13 g/L glyoxylate in shake flask cultivations. Further overexpression of glycolate oxidase and catalase significantly improved glyoxylate production to 0.74 g/L, which is the highest titer reported to date. These results demonstrate that microbial fermentation has a promising potential to manufacture glyoxylate from renewable feedstocks.

1. Introduction

Glyoxylate, or glyoxylic acid, is the smallest member of the aldehyde-acid family. It possesses both aldehyde and carboxy groups on a small molecule, thus exhibiting dual characteristics and a variety of chemical reactivity. Due to its versatility, glyoxylate is an attractive raw material for synthesizing chemicals used in perfume, pharmaceutical, agrochemical industries [1]. For example, it has been utilized as the substrate to prepare phenylglycine, allantoin, vanillin, mandelic acid, and so on [2]. Currently, the commercial production of glyoxylate employs chemical or electrochemical oxidation methods using petrochemical resources as feedstocks. Chemical oxidation usually treats glyoxal in the presence of a strong acid such as nitric acid, while electrochemical methods include the reduction of oxalic acid or anodic oxidation of glyoxal to produce glyoxylate [2]. In order to overcome the drawbacks of chemical methods, researchers have been paying increasing attention to the biotechnological production route.

Glycolate oxidase (EC:1.1.3.15) is a family of peroxisomal flavoprotein which catalyzes the oxidation of α-hydroxy acid. It widely exists in the leaves of green plants and the livers of animals [3–6]. Glycolate oxidase from spinach has been intensively studied for enzymatic production of glyoxylate. Biocatalysts employing soluble enzyme [7] or engineered microbial cells [8] have achieved high selectivity and yield transformation of glycolate to glyoxylate. In addition, the enzymatic production of glyoxylate was accompanied with hydrogen peroxide (H_2O_2) formation, which could cause glycolate oxidase deactivation and decompose glyoxylate to formate. Therefore, during the enzymatic conversion process, catalase was required to remove H_2O_2 and ethylenediamine was included to prevent further oxidation of glyoxylate [7,8]. In addition, glycolate oxidase from microorganisms including *Alcaligenes* sp. [9] was also employed for glyoxylate production, obtaining 0.65 M glyoxylate from 1.0 M glycolate by incubating at 20 °C for 15 days. Recently, a glycolate oxidase with higher specific activity than the enzyme from spinach was isolated from *Medicago falcate* [10], and immobilization of glycolate oxidase using magnetic nanoparticles was applied to enhance the enzyme stability and the optimum temperature [10,11].

As described above, the enzymatic production of glyoxylate has shown its potential for large-scale industrial application. However, the enzymatic biocatalysts still have the disadvantages that petroleum-derived glycolate is used as the substrate, and also obtaining glycolate oxidase is a time-consuming and high cost process. In this regard, a microbial fermentation routine which converts renewable biomass to glyoxylate would be more sustainable and possibly cost-effective. The Methylotrophic bacteria *Methylobacterium* sp. MB200 was reported to accumulate glyoxylate using methanol as carbon source. However, glyoxylate was

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https://doi.org/10.1016/j.bej.2017.11.002
1369-703X/

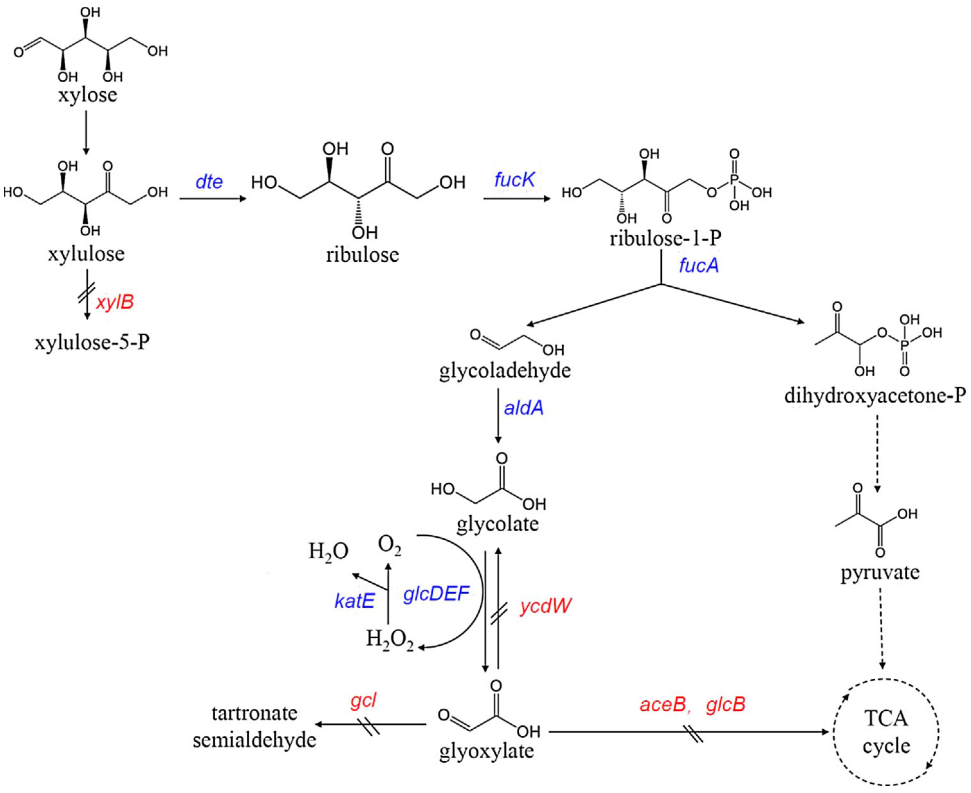

Fig. 1. Glyoxylate producing pathway from xylose in recombinant *E. coli*.
Gene annotation: *dte*, D-tagatose 3-epimerase; *fucK*, L-fuculokinase; *fucA*, L-fuculose-phosphate aldolase; *aldA*, aldehyde dehydrogenase A, NAD-linked; *glcD*, glycolate oxidase subunit, FAD-linked; *glcE*, glycolate oxidase FAD binding subunit; *glcF*, glycolate oxidase 4Fe-4S iron-sulfur cluster; *katE*, catalase HPII; *xylB*, xylulokinase; *aceB*, malate synthase A; *glcB*, malate synthase G; *gcl*, glyoxylate carboligase; *ycdW*, glyoxylate reductase.

only found to accumulated intracellularly and no production was detected in the culture medium [12].

Xylose is the second most abundant sugar in lignocellulosic biomass and is regarded as a promising substrate for microbial fermentation processes. Previously, we reported the effective production of two-carbon products including ethylene glycol and glycolate from xylose by metabolic engineering of *Eshcherichia coli* [13]. In this study, *E. coli* was further engineered to achieve glyoxylate production. The metabolic engineering strategies include inactivation of malate synthase genes *aceB*/*glcB* and glyoxylate carboligase gene *gcl*, overexpression of glycolate oxidase operon *glcDEF* and catalase gene *katE* (Fig. 1). Our results presented here provided a novel strategy for microbial glyoxylate production using xylose as feedstocks.

2. Materials and methods

2.1. Bacterial strains and plasmids

Bacterial strains, plasmids, and primers used in this study are listed in Table 1. *E. coli* K12 *ΔrecA ΔendA ΔxylB* was used as the host for strain engineering and glyoxylate producing experiments. Oligonucleotides were purchased from Sangon Biotech (Shanghai, China). Gene deletions in *E. coli* strain were performed according to the protocols reported previously [14,15]. The primers used in gene deletions are listed in Supplementary Table 1. All of the constructed *E. coli* mutants were verified by colony PCR and DNA sequencing using appropriate primers. *E. coli* JM109 was employed as the host for plasmid construction and preservation.

All DNA manipulations were performed according to standard molecular cloning procedures or manufacturers' instructions. Primers glcF/glcR were designed to amply the glycolate oxidase operon *glcDEF* from *E. coli* genomic DNA. The PCR product was digested with *Hind*III-*Xba*I and cloned into the expression vector pEn cut with the same enzymes to form pEn-glcDEF. Similarly, *katE* encoding catalase was amplified from *E. coli* genomic DNA using primers katF/katR. The PCR products were purified and digested with *Kpn*I-*Xba*I, and inserted into the corresponding restriction sites of pEn to construct pEn-katE. In addition, P_{pdc}-*katE* was excised from pEn-katE using *Spe*I/*Xho*I and cloned into pEn-glcDEF digested with *Nhe*I/*Xho*I to generate pEn-glcDEF-katE.

2.2. Culture conditions

For routine cultivations including plasmid construction and strain engineering, *E. coli* strains were cultivated with Luria-Bertani (LB) broth or on LB agar plates containing appropriate antibiotics at 37 °C. LB medium contained 5 g/L yeast extract, 10 g/L Bacto tryptone and 10 g/L NaCl. When necessary, antibiotics were added to culture medium at the following concentrations: 100 μg/mL ampicillin (Amp) and 50 μg/mL kanamycin (Kan).

To prepare the seed cultures, the strains were incubated at 37 °C in 20 mL of LB medium for 12 h at a rotation rate of 200 rpm. For glyoxylate producing shake flask studies, 5% (v/v) of the seed culture was used to inoculate into 500 mL shake flasks containing 50 mL minimal medium (MM) supplemented with 5 g/L xylose as carbon source at 37 °C and 200 rpm. MM medium comprised 2 g/L NH_4Cl, 5.0 g/L $(NH_4)_2SO_4$, 6.0 g/L KH_2PO_4, 8.4 g/L MOPS, 0.5 g/L NaCl, 0.24 g/L $MgSO_4$, 0.002 g/L Na_2MoO_4 and 1 mL/L trace element solution. The trace elements solution contained (g/L): $FeCl_2{\cdot}4H_2O$ 3.6, $CaCl_2{\cdot}2H_2O$ 5.0, $MnCl_2{\cdot}2H_2O$ 1.3, $CuCl_2{\cdot}2H_2O$ 0.38, $CoCl_2{\cdot}6H_2O$ 0.5, $ZnCl_2$ 0.94, H_3BO_3 0.0311, $Na_2EDTA{\cdot}2H_2O$ 0.4 and thiamine-HCl 1.01.