Journal of Biotechnology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Biotechnology

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

Metabolic engineering of *Corynebacterium glutamicum* for glycolate production

Ahmed Zahoor[a], Andreas Otten[b], Volker F. Wendisch[a,*]

[a] Genetics of Prokaryotes, Faculty of Biology & CeBiTec, Bielefeld University, Bielefeld, Germany
[b] Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Jülich, Jülich, Germany

ARTICLE INFO

Article history:
Received 29 October 2013
Received in revised form
18 December 2013
Accepted 20 December 2013
Available online xxx

Keywords:
Corynebacterium glutamicum
Metabolic engineering
Organic acids
Glycolate
Transcriptome

ABSTRACT

Corynebacterium glutamicum – a well-known industrial amino acid producer – has recently been engineered for the production of a variety of new products including diamines, alcohols, carotenoids and organic acids. Glycolic acid was shown here not to serve as sole or combined carbon source for *C. glutamicum*. Glycolate affected growth of *C. glutamicum* only at high concentrations (460 mM) and in a comparable manner as other salts (480 mM potassium chloride and 490 mM sodium chloride). A transcriptome analysis of cells grown in the presence of glycolate or potassium chloride revealed nine glycolate-specific gene expression changes including increased levels of a putative L-lactate permease gene when glycolate was present in medium. Subsequently, glycolate was shown to interfere with L-lactate utilization but not with growth with acetate or pyruvate. Heterologous expression of the glyoxylate reductase gene *ycdW* from *Escherchia coli* resulted in a titer of 0.4 g/L glycolate in minimal medium with glucose and acetate. Deletion of the malate synthase gene *aceB* improved glycolate titer by about tenfold. Reducing isocitrate dehydrogenase activity by replacing the translational start codon (ATG to GTG) further increased glycolate titer by more than 30%. The production of 5.3 ± 0.1 g/L glycolate with a yield of 0.18 g/g and a volumetric productivity of about 0.1 g L^{-1} h^{-1} is the first report of a *C. glutamicum* strain capable of glycolate production.

1. Introduction

Organic acids are produced annually on a multi-million-ton scale and are used in various industries including food, pharmaceutical and chemical industries (Sauer et al., 2008). Many of the organic acids are currently produced from petrochemical sources employing chemical routes that neither are renewable nor environmental friendly (Hatti-Kaul et al., 2007). On the other hand, organic acids represent one of the major classes of compounds produced by microorganisms. Besides a plethora of natural acid producers, e.g. for lactic and citric acid, bacterial strains such as *Escherichia coli* and *Corynebacterium glutamicum* have been metabolically engineered for the production of organic acids (Wendisch et al., 2006; Wieschalka et al., 2013; Zahoor et al., 2012).

Glycolate ($HOCH_2COOH$) is the simplest alpha-hydroxy acid. It finds multiple applications e.g. in the cosmetic industry to improve skin texture and to treat skin diseases (Rendon et al., 2008). As it complexes metal ions glycolate is used as a cleaning and rinsing agent in household and industrial applications. Polymerization of glycolate alone or with other acids such as L-lactate yields polymers with excellent gas barrier properties making them ideal for manufacturing of packaging materials. Since glycolate polymers hydrolyze at controllable rates in an aqueous environment they are suited for medical applications (Fredenberg et al., 2011) such as in dissolvable sutures.

Glycolate is chemically manufactured by high-pressure, high-temperature carbonylation of formaldehyde (Loder, 1939) or by enzymatic conversion of glycolonitrile using microbial nitrilases (He et al., 2010; Panova et al., 2007), however, glycolonitrile and its degradation products formaldehyde and hydrogen cyanide are toxic. Bioconversion of ethylene glycol to glycolate by yeasts and bacteria such as *Gluconobacter oxydans* has also been reported (Kataoka et al., 2001; Wei et al., 2009). *E. coli* was metabolically engineered for glycolate production with titers up to 1.4 g/L by overexpression of genes coding for glyoxylate reductase, isocitrate lyase and isocitrate dehydrogenase kinase/phosphatase (Dischert and Soucaille, 2012; Martin et al., 2013; Soucaille, 2009). Glycolate titers up to 15 g/L at low pH have also been recently reported for yeasts (Koivistoinen et al., 2013).

C. glutamicum is a Gram-positive bacterium with a history of 50 years of safe production in the food and feed industries. Originally isolated natural glutamate producer (Kinoshita et al., 1957) it is currently used for the annual production of 2,160,000 tons of

* Corresponding author at: Genetics of Prokaryotes, Faculty of Biology & CeBiTec, University of Bielefeld, 33615 Bielefeld, Germany. Tel.: +49 521 106 5611.
E-mail address: volker.wendisch@uni-bielefeld.de (V.F. Wendisch).

0168-1656/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2013.12.020

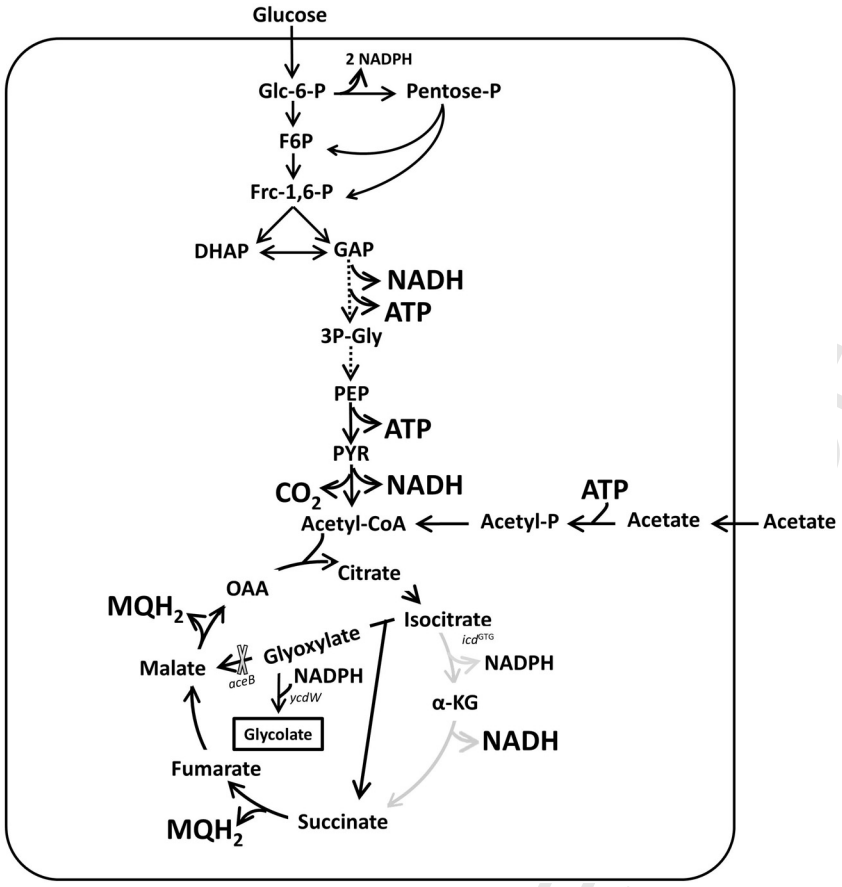

Fig. 1. Schematic representation of the central carbon metabolism of *C. glutamicum* and metabolic engineering targets for glycolate production. The glyoxylate reductase gene *ycdW* from *E. coli* was heterologously expressed. The malate synthase gene (*aceB*) was deleted to block conversion of glyoxylate to malate. The translational start codon of the isocitrate dehydrogenase gene *icd* was changed to GTG in the genome to reduce isocitrate conversion in the tricarboxylic acid cycle. Dotted arrows represent multiple reactions. Glc-6-P, glucose 6 phosphate; Frc-1,6-P, fructose 6 phosphate; DHAP, dihydroxyacetonephosphate; GAP, glyceraldehyde-3-phosphate; 3-P Gly, 3-phosphoglycerate; PEP, phosphoenolpyruvate; PYR, pyruvate; α-KG, α-ketoglutarate; OAA, oxaloacetate.

glutamate (Ajinomoto, 2010) and 1,480,000 tons of lysine (Ajinomoto, 2011). The availability of genetic tools for *C. glutamicum*, its genome sequence (Ikeda and Nakagawa, 2003; Kalinowski et al., 2003) and omics technologies such as transcriptomics (Wendisch, 2003) led to metabolic engineering for the production of a various amino acids, organic acids, alcohols, diamines and carotenoids (Zahoor et al., 2012). Examples are serine (Stolz et al., 2007) and proline (Jensen and Wendisch, 2013) succinate (Litsanov et al., 2012a,b), pyruvate (Wieschalka et al., 2012), lactate (Okino et al., 2005, 2008), α-ketoglutarate (Jo et al., 2012), 2-ketoisovalerate (Krause et al., 2010), isobutanol (Blombach et al., 2011; Smith et al., 2010), pantothenate (Chassagnole et al., 2003; Hüser et al., 2005), 1,5-diaminopentane (Kind et al., 2011; Mimitsuka et al., 2007), 1,4-diamonobutane (Schneider et al., 2012; Schneider and Wendisch, 2010) and various carotenoids (Heider et al., 2012, 2013). In parallel, access to pentoses in hydrolysates (Gopinath et al., 2012; Meiswinkel et al., 2013) crude glycerol (Meiswinkel et al., 2013; Rittmann et al., 2008), cellubiose (Adachi et al., 2013), starch (Song et al., 2013) and amino sugars (Uhde et al., 2013) has been engineered to realize a flexible feedstock concept for sustainable production.

Here we characterize the response of *C. glutamicum* to glycolate and report metabolic engineering for glycolate production up to 5.3 g/L in batch cultivations. The central metabolism of *C. glutamicum* (Fig. 1) was extended by glyoxylate reductase from *E. coli* since the *C. glutamicum* lacks the respective gene. To increase precursor supply the malate synthase gene *aceB* was deleted and expression the isocitrate dehydrogenase gene *icd* reduced by changing the translational start codon from ATG to GTG.

2. Materials and methods

2.1. Strains and plasmids

C. glutamicum ATCC 13032 was used in this study. All strains and plasmids used in this study are mentioned in Table 1; the primers used are listed in Table 2. *E. coli* and *C. glutamicum* strains were routinely grown in lysogeny broth (LB) at 37 °C and 30 °C, respectively, in 500-mL baffled flasks on a rotary shaker at 120 rpm. CGXII minimal medium (Keilhauer et al., 1993) was used for *C. glutamicum* and growth was followed by measuring optical density at 600 nm. Where necessary, the growth medium was supplemented with kanamycin (25 μg/mL), spectinomycin (50 μg/mL) and isopropyl β-D-1-thiogalactopyranoside (IPTG) (1 mM). Glycolate was bought from Sigma–Aldrich (product # 124737) and a stock solution was prepared with neutralized pH for use in experiments.

2.2. Standard DNA work

The primers were obtained from EurofinsDNA (Ebersberg, Germany) or from Metabion (Martinsried, Germany). Standard methods such as PCR, restriction digestion and ligation were carried out according to manufacturer's manual. *E. coli* competent cells were transformed by heat shock method (Sambrook and Russell,

Please cite this article in press as: Zahoor, A., et al., Metabolic engineering of *Corynebacterium glutamicum* for glycolate production. J. Biotechnol. (2014), http://dx.doi.org/10.1016/j.jbiotec.2013.12.020