Journal of Biotechnology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Biotechnology

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

Engineering biotin prototrophic *Corynebacterium glutamicum* strains for amino acid, diamine and carotenoid production

P. Peters-Wendisch*, S. Götker, S.A.E. Heider, G. Komati Reddy, A.Q. Nguyen, K.C. Stansen, V.F. Wendisch

Genetics of Prokaryotes, Faculty of Biology and CeBiTec, Bielefeld University, Bielefeld, Germany

ARTICLE INFO

Article history:
Received 5 November 2013
Received in revised form
21 December 2013
Accepted 3 January 2014
Available online xxx

Keywords:
Corynebacterium glutamicum
Biotin biosynthesis
Amino acid production
Diamine production
Carotenoid production

ABSTRACT

The Gram-positive *Corynebacterium glutamicum* is auxotrophic for biotin. Besides the biotin uptake system BioYMN and the transcriptional regulator BioQ, this bacterium possesses functional enzymes for the last three reactions of biotin synthesis starting from pimeloyl-CoA. Heterologous expression of *bioF* from the Gram-negative *Escherichia coli* enabled biotin synthesis from pimelic acid added to the medium, but expression of *bioF* together with *bioC* and *bioH* from *E. coli* did not entail biotin prototrophy. Heterologous expression of *bioWAFDBI* from *Bacillus subtilis* encoding another biotin synthesis pathway in *C. glutamicum* allowed for growth in biotin-depleted media. Stable growth of the recombinant was observed without biotin addition for eight transfers to biotin-depleted medium while the empty vector control stopped growth after the first transfer. Expression of *bioWAFDBI* from *B. subtilis* in *C. glutamicum* strains overproducing the amino acids L-lysine and L-arginine, the diamine putrescine, and the carotenoid lycopene, respectively, enabled formation of these products under biotin-depleted conditions. Thus, biotin-prototrophic growth and production by recombinant *C. glutamicum* were achieved.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Corynebacterium glutamicum is a Gram-positive bacterium originally isolated due to its ability to secrete L-glutamate (Kinoshita et al., 1957). This bacterium became the workhorse of amino acid production and is known for safe production in the food and feed industries since more than fifty years. Currently, 2,160,000 tons of L-glutamate and 1,480,000 tons of L-lysine are produced annually (Ajinomoto, 2013). *C. glutamicum* is not only used for biotechnological production of L-glutamate and L-lysine, but has also been engineered for the production of other amino acids such as L-serine (Stolz et al., 2007) and L-proline (Jensen and Wendisch, 2013), organic acids such as pyruvate (Wieschalka et al., 2012), lactate (Okino et al., 2008a,b), succinate (Litsanov et al., 2012a,b), α-ketoglutarate (Jo et al., 2012), 2-ketoisovalerate (Krause et al., 2010) and 2-ketoisocaproate (Buckle-Vallant et al., 2013). Moreover, the diamines cadaverine and putrescine (Mimitsuka et al., 2007; Schneider and Wendisch, 2010), carotenoids (Heider et al., 2012, 2014), the vitamin pantothenate (Chassagnole et al., 2003; Hüser et al., 2005) and the alcohols ethanol and isobutanol (Blombach et al., 2011; Sakai et al., 2007; Smith et al., 2010) can be produced by recombinant *C. glutamicum* strains.

C. glutamicum grows aerobically on a variety of carbon sources such as the sugars glucose, fructose and sucrose, the sugar alcohols mannitol and inositol, the organic acids gluconate, citrate, acetate, propionate, pyruvate, D-lactate and L-lactate or aromatic compounds like vanillate or protocatechuate (Blombach and Seibold, 2010; Chaudhry et al., 2007; Frunzke et al., 2008; Ikeda, 2012; Krings et al., 2006; Laslo et al., 2012; Merkens et al., 2005; Netzer et al., 2004). Industrially, mostly starch hydrolysates and molasses are used as carbon sources. Direct access to starch and glucans (Seibold et al., 2006; Tsuchidate et al., 2011) as well as to crude glycerol (Meiswinkel et al., 2013b; Rittmann et al., 2008), amino sugars (Uhde et al., 2013) or cellubiose (Adachi et al., 2013) and pentoses in cellulosic hydrolysates (Gopinath et al., 2011; Meiswinkel et al., 2013a) has been realized.

Simple mineral salts media can be used for growth of *C. glutamicum* and growth can be stimulated by iron chelators such as

* Corresponding author at: Genetics of Prokaryotes, Bielefeld University, P.O. Box 100131, D-33501 Bielefeld, Germany. Tel.: +49 521 106 5606; fax: +49 521 106 5626.
E-mail addresses: petra.peters-wendisch@uni-bielefeld.de, volker.wendisch@uni-bielefeld.de (P. Peters-Wendisch).

http://dx.doi.org/10.1016/j.jbiotec.2014.01.023
0168-1656/© 2014 Elsevier B.V. All rights reserved.

protocatechuic acid. Since *C. glutamicum* is auxotrophic for biotin (vitamin H, B7 or coenzyme R), a source of biotin has to be present in growth media. Biotin is imported into the *C. glutamicum* cell by BioYMN (Schneider et al., 2012b). The biotin requirement of *C. glutamicum* is due to only two proteins requiring this cofactor, namely pyruvate carboxylase and acetyl-CoA carboxylase (Gande et al., 2004, 2007; Jager et al., 1996; Peters-Wendisch et al., 1996, 1997, 1998, 2001; Sato et al., 2008). Both pyruvate carboxylase as the major anaplerotic carboxylase of *C. glutamicum* and acetyl-CoA carboxylase as essential for fatty acid synthesis are biotinylated at a particular lysyl residue by biotin–protein ligase BirA (Peters-Wendisch et al., 2012). Biotin starvation of *C. glutamicum* elicits L-glutamate production (Udaka, 1960). Therefore, production of other amino acids such as L-lysine by *C. glutamicum* requires provision of sufficient biotin (Ko and Chipley, 1984), in particular since biotin-dependent pyruvate carboxylase is a bottleneck of lysine production (Peters-Wendisch et al., 2001).

Biotin-auxotrophic *C. glutamicum* surprisingly possesses not only BioYMN for biotin uptake and BirA for protein biotinylation (Fig. 1) (Peters-Wendisch et al., 2012; Schneider et al., 2012b), but also biotin transcriptional regulator BioQ (Brune et al., 2012) and the enzymes for the three final reactions of biotin synthesis, 7,8-diaminononanoate synthase BioA, dethiobiotin synthase BioD and biotin synthase BioB (Hatakeyama et al., 1993a,b; Hatakeyama et al., 1997). Biotin synthesis can be divided into two parts: synthesis of pimelate thioester (pimeloyl-CoA or pimeloyl-acyl carrier protein (ACP)) followed by the biotin ring assembly (Lin and Cronan, 2011). Of the four highly conserved reactions of biotin ring assembly (Streit and Entcheva, 2003) only 8-amino-7-oxononanoate synthase BioF is not encoded in the *C. glutamicum* genome (Kalinowski et al., 2003). Since desthiobiotin and aminopelargonic acid derivatives, but not pimelic acid, supported growth when added in low concentrations (Okumura et al., 1962), biotin auxotrophy of *C. glutamicum* was considered to be due to the lack of BioF (Hatakeyama et al., 1993a,b; 1997). Alternatively, *C. glutamicum* might lack BioF and in addition a biosynthetic pathway leading to its substrate pimeloyl-CoA/ACP (Fig. 1).

Two distinct major pathways for biosynthesis of pimeloyl-CoA/ACP are known (Fig. 1), the BioC-BioH pathway in *E. coli* and the BioI-BioW pathway in *Bacillus subtilis* (Lin and Cronan, 2011). In *E. coli*, S-adenosyl methionine-dependent methyltransferase BioC methylates malonyl-CoA and the resulting methyl ester enters fatty acid biosynthesis (Lin and Cronan, 2012). After two elongation cycles carboxylesterase BioH demethylates the generated methyl-pimeloyl-ACP to pimeloyl-ACP, the substrate of biotin ring assembly (Agarwal et al., 2012). In *B. subtilis*, the P450 protein BioI oxidatively cleaves a carbon-carbon bond of an acyl-ACP intermediate of fatty acid biosynthesis to generate pimeloyl-ACP (Cryle and Schlichting, 2008; Green et al., 2001; Lin and Cronan, 2011). Alternatively, pimeloyl-CoA synthetase BioW from *B. subtilis* activates externally added pimelic acid (Bower et al., 1996) to yield pimeloyl-CoA as precursor for biotin ring assembly.

In order to render *C. glutamicum* biotin prototroph we initiated experiments regarding heterologous expression of genes either of the *E. coli* BioC-BioH pathway as well as of the *B. subtilis* BioI-BioW pathway. Here we show that expression of *bioF* from *E. coli* allows for biotin synthesis from pimelic acid added to the medium, but expression of *bioF* alone or together with *bioC* and *bioH* from *E. coli* did not entail biotin prototrophy. By contrast, expression of the complete biotin synthesis operon *bioWAFDBI* from *B. subtilis* converted *C. glutamicum* to a biotin prototroph. Very recently, a similar approach was used to construct biotin hyper-auxotrophic and prototrophic *C. glutamicum* strains (Ikeda et al., 2013).

2. Materials and methods

2.1. Bacterial strains, plasmids, oligonucleotides, and culture conditions

Bacterial strains and plasmids used are listed in Table 1. *Escherichia coli*, *Bacillus subtilis* and *C. glutamicum* were grown in lysogeny broth complex medium (LB) as the standard medium (Sambrook and Russell, 2001) and kanamycin (25 μg/l) and/or spectinomycin (100 μg/l) were added when appropriate. The minimal medium used for growth and amino acid production by *C. glutamicum* was described previously (Keilhauer et al., 1993) and contained 100 mM or 220 mM glucose and 0–200 μg biotin l^{-1}, respectively. The cultures (50 ml in 500 ml baffled Erlenmeyer flasks) were inoculated to give an optical density at 600 nm (OD_{600}) of about 1 and then incubated aerobically at 30 °C on a rotary shaker at 120 rpm. For induction of the expression either 10 μM, 50 μM or 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) were added to the cultures.

2.2. Construction of plasmids and strains

Standard methods such as PCR, restriction, and ligation were carried out as described previously (Sambrook and Russell, 2001). Plasmids were constructed in *Escherichia coli* DH5α from PCR-generated fragments (KOD, Novagen) and isolated with the QIAprep spin miniprep kit (QIAGEN, Hilden, Germany). All cloned DNA fragments were shown to be correct by sequencing.

For the construction of the expression vectors pEKEX3*bioF* and pEKEX3bio*FCH* chromosomal DNA from *E. coli* K-12 MG1655 and for the construction of pEKEX3*bioWAFDBI* chromosomal DNA from *B. subtilis* sub. *subtilis* str. 168 was isolated as described (Eikmanns et al., 1994). The oligonucleotides used for the PCR amplification of *bioF*, *bioC* and *bioH* from *E. coli* and *bioWAFDBI* from *B. subtilis* as well as for the construction of pVWEx1 are listed in Table 1 and were obtained from Metabion (Martinsried, Germany) or Life Technologies (Carlsbad, CA, USA). The *bioWAFDBI* operon was amplified from genomic DNA of *B. subtilis* sub. *subtilis* str. 168 using the primers bioWAFDBI-fw and bioWAFDBI-rv (Table 1). The PCR product was cloned into pEKEx3 (Stansen et al., 2005) using the restriction sites introduced by the primers to yield pEKEx3-*bioWAFDBI*. Some transformants showed irregular plasmids and were not able to grow without biotin which might be due to homologous recombination between *bioA*, *bioB* or *bioD* on the chromosome and on the plasmid. Plasmid pVWEx1-$argB_{A26VM31V}$ was constructed by digesting pEKEx3-$argB_{A26VM31V}$ (Schneider et al., 2011) with KpnI and BamHI and ligation of the resulting fragment $argB_{A26VM31V}$ into KpnI/BamHI digested pVWEx1 vector. The resulting plasmid pVWEx1-$argB_{A26VM31V}$ was verified by sequencing. Transformation of *E. coli* was performed using the calcium chloride method (Sambrook and Russell, 2001), while *C. glutamicum* was transformed by electroporation as described previously (Eggeling and Reyes, 2005). The resulting strains are listed in Table 1.

2.3. Quantification of amino acid, polyamine and lycopene concentrations

For quantification of extracellular amino acids and polyamines, aliquots of the culture were withdrawn, the optical density (OD_{600}) was measured and cells were removed by centrifugation (13,000 × g, 10 min). The supernatant was analyzed using a high-pressure liquid chromatography system (HPLC, 1200 series, Agilent Technologies Deutschland GmbH, Böblingen, Germany).

Amino acids were determined by automatic precolumn derivatization with *ortho*-phthaldialdehyde as described previously (Georgi et al., 2005) and separated on a reversed phase

Please cite this article in press as: Peters-Wendisch, P., et al., Engineering biotin prototrophic *Corynebacterium glutamicum* strains for amino acid, diamine and carotenoid production. J. Biotechnol. (2014), http://dx.doi.org/10.1016/j.jbiotec.2014.01.023

Download English Version:

https://daneshyari.com/en/article/6491286

Download Persian Version:

https://daneshyari.com/article/6491286

Daneshyari.com