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The pyruvate dehydrogenase complex of *Corynebacterium glutamicum*: An attractive target for metabolic engineering

Bernhard J. Eikmanns [a], Bastian Blombach [b,*]

[a] Institute of Microbiology and Biotechnology, University of Ulm, 89069 Ulm, Germany
[b] Institute of Biochemical Engineering, University of Stuttgart, 70569 Stuttgart, Germany

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ABSTRACT

The pyruvate dehydrogenase complex (PDHC) catalyzes the oxidative thiamine pyrophosphate-dependent decarboxylation of pyruvate to acetyl-CoA and CO_2. Since pyruvate is a key metabolite of the central metabolism and also the precursor for several relevant biotechnological products, metabolic engineering of this multienzyme complex is a promising strategy to improve microbial production processes. This review summarizes the current knowledge and achievements on metabolic engineering approaches to tailor the PDHC of *Corynebacterium glutamicum* for the bio-based production of L-valine, 2-ketosiovalerate, pyruvate, succinate and isobutanol and to improve L-lysine production.

1. Introduction

Industrial biotechnology is regarded as one of the current key technologies with an expected market volume of more than $300 billion by 2030, only considering microbial, non-biopharmaceutical products (Festel, 2010; Neubauer, 2011). Limited fossil resources represent the main driver for the development of cost effective processes for the production of chemicals from renewable feedstocks. Microbial fermentation is an established industrial applicable technology and advances more and more to an economic feasible alternative to crude-oil based production processes (Bozell and Petersen, 2010). Accordingly, the portfolio of biotechnological products increases rapidly (reviewed in Straathof, 2013). These achievements are occasionally possible due to the steadily increasing knowledge on metabolism and pathway regulation of industrial relevant organisms. However, metabolic engineering approaches very often represent defined applications for one target product and are not generally suited for other applications or further target products. Most relevant microbial products (native or not) derive from central metabolites, i.e., share a common precursor such as acetyl-CoA, oxaloacetate or pyruvate. Therefore, engineering the central metabolism is attractive not only for a certain product but moreover for the complete product class derived from the given central metabolite.

Pyruvate is a central intermediate of all organisms and represents the turntable distributing carbon into amino acid synthesis, the citric acid cycle (oxidative or reductive), fatty acid synthesis, anaplerosis or is used for regeneration of NAD^+ by the formation of lactate under anaerobic conditions. Under ordinary aerobic growth conditions, a major pyruvate converting enzyme in most of the known microorganisms is the pyruvate dehydrogenase complex (PDHC), which represents an attractive target for metabolic engineering.

Corynebacterium glutamicum is a Gram-positive facultative anaerobic organism that grows on a variety of sugars, organic acids, and alcohols as single or combined carbon and energy sources (Eggeling and Bott, 2005; Liebl, 2006; Nishimura et al., 2007; Takeno et al., 2007). The organism is generally regarded as safe (GRAS status) and is the workhorse for large scale production of amino acids, such as L-glutamate (world-wide production by fermentation > 2.5 million t/a) and L-lysine (>1.9 million t/a) (Eggeling and Bott, 2005; Takors et al., 2007; Ajinomoto, 2013).

* Corresponding author at: Institute of Biochemical Engineering, University of Stuttgart, Allmandring 31, 70569 Stuttgart, Germany. Tel.: +49 711 685 64549; fax: +49 711 685 65164.
E-mail address: blombach@ibvt.uni-stuttgart.de (B. Blombach).

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In order to improve the production performance by metabolic engineering approaches, the central carbon metabolism, the physiology and the regulation of relevant pathways of *C. glutamicum* were analyzed in detail and genetic tools as well as systems biology approaches on the 'omics' level have been developed and employed (overviews in Kirchner and Tauch, 2003; Eggeling and Bott, 2005; Sauer and Eikmanns, 2005; Wendisch et al., 2006; Bott, 2007; Takors et al., 2007; Burkowski, 2008; Brinkrolf et al., 2010; Becker and Wittmann, 2011; Teramoto et al., 2011; Vertès et al., 2012). Recent studies explored the usefulness of *C. glutamicum* for the production of other commodity chemicals, such as the biofuels isobutanol and ethanol (Inui et al., 2004; Smith et al., 2010; Blombach and Eikmanns, 2011; Blombach et al., 2011), the diamines cadaverine and putrescine (Mimitsuka et al., 2007; Schneider and Wendisch, 2010, 2011; Kind et al., 2010a,b; Kind and Wittmann, 2011), the sugar alcohol xylitol (Sasaki et al., 2010), gamma-amino butyric acid (Takahashi et al., 2012), polyhydroxybutyrate (Song et al., 2012), the chemical chaperone ectoine (Becker et al., 2013), carotenoids (Heider et al., 2012, 2013) and also several organic acids such as succinate, D-lactate and 2-ketoisocaproate (reviewed in Wieschalka et al., 2012a; Bückle-Vallant et al., 2013).

Since the common precursor of several products mentioned above is pyruvate, the optimization of its availability has a high potential to improve microbial production processes. Despite the major importance of pyruvate as precursor for relevant biotechnological products and the central role of the PDHC in the metabolism of industrially important organisms, only the PDHC of *C. glutamicum* was intensively engineered to establish a microbial platform with completely abolished or with reduced PDHC activity useful for the bio-based production of not only pyruvate but also for its derived products L-lysine, succinate, 2-ketoisovalerate, L-valine and isobutanol (Fig. 1). This review summarizes the current knowledge and

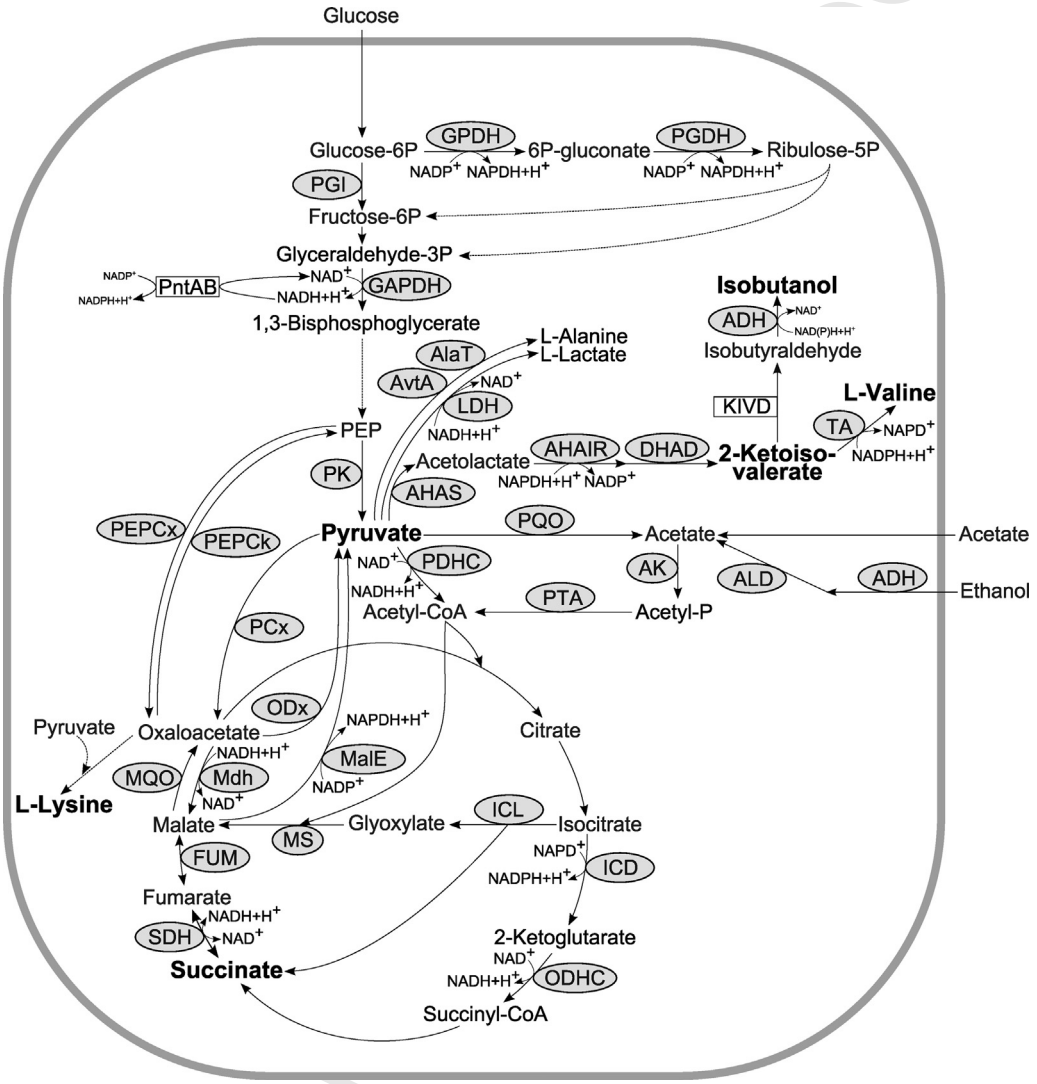

Fig. 1. Schematic presentation of the central carbon metabolism of *C. glutamicum* including pathways for the production of pyruvate, 2-ketoisovalerate, succinate, L-lysine, L-valine and isobutanol (not native). Ellipses represent proven enzymes present in *C. glutamicum*. Rectangles represent heterologous enzymes. *Abbreviations*: coding genes are given in brackets. ADH (*adhA*), alcohol dehydrogenase A; AHAIR (*ilvC*), acetohydroxyacid isomeroreductase; AHAS (*ilvBN*), acetohydroxyacid synthase; AK (*ack*), acetate kinase; AlaT (*alaT*), alanine aminotransferase; ALD (*ald*), acetaldehyde dehydrogenase; AvtA (*avtA*), valine-pyruvate aminotransferase; DHAD (*ilvD*), dihydroxyacid dehydratase; FUM (*fum*), fumarase; GAPDH (*gapA*), glyceraldehyde-3P dehydrogenase; GPDH (*zwf*, *opcA*), glucose-6P dehydrogenase; ICD (*icd*), isocitrate dehydrogenase; ICL (*aceA*), isocitrate lyase; KIVD, 2-ketoacid decarboxylase from *L. lactis*; LDH (*ldhA*), L-lactate dehydrogenase; MalE (*malE*), malic enzyme; Mdh (*mdh*), malate dehydrogenase; MQO (*mqo*), malate:quinone oxidoreductase; MS (*aceB*), malate synthase; ODHC (*odhA*, *aceF*, *lpd*), 2-ketoglutarate dehydrogenase complex; ODx (*odx*), oxaloacetate decarboxylase; PCx (*pyc*), pyruvate carboxylase; PDHC (*aceE*, *aceF*, *lpd*), pyruvate dehydrogenase complex; PEP phosphoenolpyruvate; PEPCk (*pck*), PEP carboxykinase; PEPCx (*ppc*), PEP carboxylase; PGDH (*gnd*), 6P-gluconate dehydrogenase; PGI (*pgi*), phosphoglucose isomerase; PK (*pyk*), pyruvate kinase; PntAB (*pntAB*), membrane bound transhydrogenase from *E. coli*; PQO (*pqo*), pyruvate:quinone oxidoreductase; PTA (*pta*), phosphotransacetylase; SDH (*sdhABC*), succinate dehydrogenase; TA (*ilvE*), transaminase B.

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