Bioresource Technology 239 (2017) 412–421

Contents lists available at ScienceDirect

Bioresource Technology

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

Protein and metabolic engineering for the production of organic acids

Jingjing Liu [a,b], Jianghua Li [a,b,*], Hyun-dong Shin [c], Long Liu [a,b], Guocheng Du [a,b], Jian Chen [b]

[a] Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, Jiangnan University, Wuxi 214122, China
[b] Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University, Wuxi 214122, China
[c] School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta 30332, USA

HIGHLIGHTS

- The types and applications of main organic acids were overviewed.
- Production of organic acids by protein and metabolic engineering strategies were summarized.
- The future prospects for organic acids production using systems and synthetic biology were discussed.

ARTICLE INFO

Article history:
Received 24 January 2017
Received in revised form 10 April 2017
Accepted 12 April 2017
Available online 14 April 2017

Keywords:
Organic acid
Carboxylic acid
Protein
Metabolic engineering
Biosynthetic pathways

ABSTRACT

Organic acids are natural metabolites of living organisms. They have been widely applied in the food, pharmaceutical, and bio-based materials industries. In recent years, biotechnological routes to organic acids production from renewable raw materials have been regarded as very promising approaches. In this review, we provide an overview of current developments in the production of organic acids using protein and metabolic engineering strategies. The organic acids include propionic acid, pyruvate, itaconic acid, succinic acid, fumaric acid, malic acid and citric acid. We also expect that rapid developments in the fields of systems biology and synthetic biology will accelerate protein and metabolic engineering for microbial organic acid production in the future.

1. Introduction

The petrochemical industry affects almost every aspect of our daily lives. Throughout the past century, a variety of valuable chemicals derived mainly from fossil resources have been introduced, and the market for these products continues to grow (Becker and Wittmann, 2015). However, public concerns over environmental pollution, greenhouse gas emissions, and the shortage of raw oils are increasing, and attention is turning to alternative, renewable sources of chemical products to reduce both dependency on oil reserves and carbon dioxide emissions into the environment (Arslan et al., 2012). Organic acids, especially carboxylic acids, can be used as substitutes for these products because they are essential intermediate metabolites in cells (Wang et al., 2016). They have been applied in a wide range of industries, including food, pharmaceutical, cosmetic, detergent, polymer, and textile (Becker et al., 2015).

In the last decade, there have been many studies on the biosynthesis of organic acids, and various types of enzymes and microorganisms have been developed for this purpose (Alonso et al., 2015). Low cost, low energy and high yield are desirable properties in organic acid biosynthesis. Free or immobilized enzymes, whole-cell catalysts, and direct fermentation by natural or recombinant strains have been applied for industrial production. *Escherichia coli*, a typical example of a microbial cell factory, is one of the most frequently used prokaryotic expression systems and is the most suitable laboratory model for research because its genome has been characterized and its growth requirements and short doubling time are economical and feasible for studies (Zajkoska et al., 2013). Purified proteins from *E. coli* can be used directly for substrate catalysis, and immobilized enzymes or whole cells can be used as tools to create economical and ecological biocatalysts. Based on metabolic engineering of glycolysis, Krebs cycle, and pentose phosphate pathway networks, *E. coli* can be used to produce various organic acids (Yu et al., 2011). The intensive efforts to develop strains and related enzymes and processes in recent years have contributed to our current understanding of organic acid production and process optimization (Becker and Wittmann, 2015; Becker et al., 2015).

* Corresponding author at: Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, Jiangnan University, Wuxi 214122, China.
E-mail address: lijianghua@jiangnan.edu.cn (J. Li).

http://dx.doi.org/10.1016/j.biortech.2017.04.052
0960-8524/

In this review, we divided organic acids into three types: monocarboxylic acids, dicarboxylic acids, and multi-carboxylic acids. For each class, we summarize the progress over the past decade in the biological production of valuable organic acids, including PA, pyruvate, itaconic acid (IA), succinic acid (SA), FA, malic acid (MA), citric acid (CA), and ICA. Finally, we discuss further improvements needed for the production of organic acids, using protein engineering, systems biology, and synthetic biology strategies.

2. Monocarboxylic acids

2.1. Propionic acid

PA is an important C-3 platform chemical with a variety of applications in the food, perfume, paint, and pharmaceutical industries (Liu et al., 2012). PA is among the top 30 candidate platform chemicals produced from biomass, according to the US Department of Energy (Werpy and Petersen, 2004). Its calcium, sodium, and ammonium salts are commonly used to preserve foods and animal feed because of their antimicrobial characteristics (Guan et al., 2016).

As commonly used PA producers, propionibacteria can utilize a wide range of carbon sources and are generally recognized as safe (Guan et al., 2015). *Propionibacterium thoenii*, *Propionibacterium jensenii*, *Propionibacterium freudenreichii*, and *Propionibacterium acidipropionici* are propionibacteria species from dairy products and have been used for PA production.

2.1.1. Dicarboxylic acid pathway engineering for enhanced PA production

Propionibacteria produce PA through the dicarboxylic acid pathway (Fig. 1), with lactate and acetic acid as the main by-products. Glycerol has been explored as a favorable carbon source for PA production, because less acetic acid is produced during fermentation (Zhu et al., 2010). Overexpression of glycerol dehydrogenase (GDH) can not only accelerate glycerol dissimilation but also generate NADH in the process (Xin et al., 2013; Long et al., 2015). With key metabolic nodes that limit PA overproduction in *P. jensenii*, co-overexpression of GDH and malate dehydrogenase (MDH) enhanced the PA titer to 39.43 g/L (Long et al., 2015).

Phosphoenolpyruvate carboxylase (PPC), pyruvate carboxylase (PYC), methylmalonyl-CoA decarboxylase (MMD), and methylmalonyl-CoA carboxyltransferase (MMC) are critical carboxylases in the dicarboxylic acid pathway that control carbon flux in the Wood–Werkman cycle. By expressing PPC, MMD, and MMC to increase carbon flux toward oxaloacetate (OAA) in the dicarboxylic acid pathway, the recombinant *Propionibacterium* grew significantly faster and the PA titer was significantly enhanced (Ammar et al., 2014; Wang et al., 2015b).

2.1.2. Improving the productivity of PA with FBB-immobilized cells

Fermentative production of PA is more expensive than other chemicals, because strong product inhibition results in a low PA titer. Therefore, how to enhance the tolerance of cells for PA has been a major issue (Zhang and Yang, 2009). The fibrous bed bioreactor (FBB) system, is one of the most promising bioprocess to produce organic acids (Wallenius et al., 2015).

An acetate kinase knock-out strain, *P. acidipropionici* ACK-Tet, was immobilized and adapted to a FBB to increase its acid tolerance and acid-producing capacity. After about 3 months of adaptation, the insensitive mutant showed high Ht-ATPase activity and significantly elongated rod morphology, and the maximum PA concentration in the fermentation broth reached 100 g/L (Zhang and Yang, 2009). Continuous PA production from renewable carbon sources such as sugarcane bagasse hydrolysate or whey lactose can be achieved, by a developed FBB with immobilized *P. acidipropionici* in the reactor (Zhu et al., 2012; Jiang et al., 2015). The highest titer of PA reached 135 ± 6.5 g/L in a fed-batch fermentation of whey lactose with *P. acidipropionici* immobilized on polyethylenimine-treated Poraver (PEI-Poraver) in FBB (Jiang et al., 2015).

Besides adaptive evolution and genome shuffling, metabolic engineering of *Propionibacteria* was conducted to improve acid resistance and PA production. Guan and colleagues identified two key acid resistance elements, arginine deaminase and glutamate decarboxylase, and by introducing their five encoding genes (*arcA*, *arcC*, *gadB*, *gdh*, and *ybaS*) into *P. jensenii*, the PA resistance of *P. jen-*

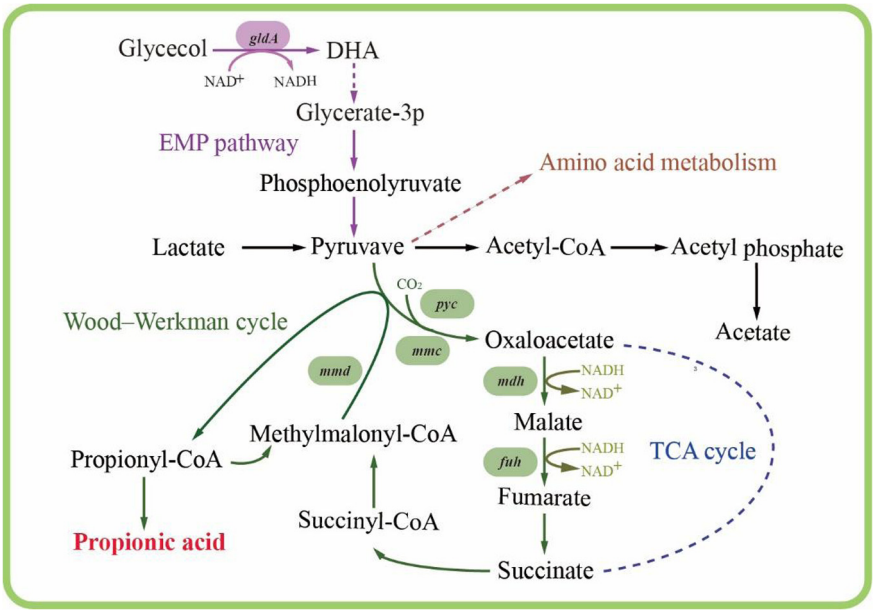

Fig. 1. Metabolic pathways for propionic acid from glycerol in *Propionibacterium*. EMP, Embden-Meyerhof-Parnas; TCA, tricarboxylic acid; *glaA*, glycerol dehydrogenase; *mdh*: malic dehydrogenase; *fuh*: fumarate hydratase; *mmc*, methylmalonyl-CoA carboxyltransferase; *mmd*, methylmalonyl-CoA decarboxylase.