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Production of succinic acid by metabolically engineered microorganisms

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Succinic acid (SA) has been recognized as one of the most important bio-based building block chemicals due to its numerous potential applications. For the economical bio-based production of SA, extensive research works have been performed on developing microbial strains by metabolic engineering as well as fermentation and downstream processes. Here we review metabolic engineering strategies applied for bio-based production of SA using representative microorganisms, including *Saccharomyces cerevisiae, Pichia kudriavzevii, Escherichia coli*, *Mannheimia succiniciproducens*, *Basfia succiniciproducens, Actinobacillus succinogenes*, and *Corynebacterium glutamicum*. In particular, strategies employed for developing engineered strains of these microorganisms leading to the best performance indices (titer, yield, and productivity) are showcased based on the published papers as well as patents. Those processes currently under commercialization are also analyzed and future perspectives are provided.

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Introduction

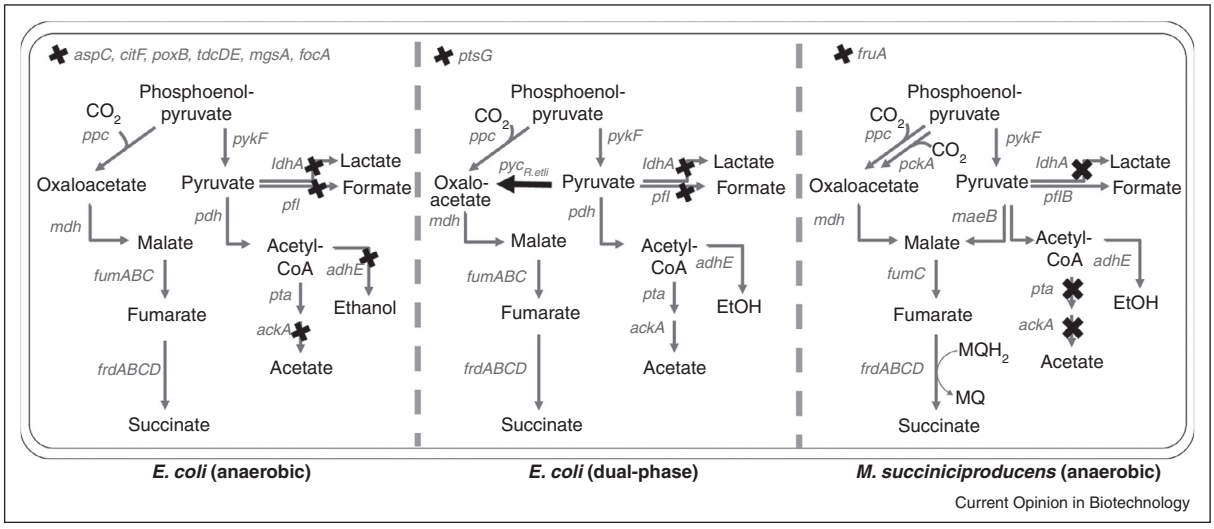
Succinic acid (SA), a four carbon dicarboxylic acid currently produced by chemical conversion of maleic anhydride [1] is an almost ubiquitous metabolite in many organisms, and thus can be produced by microbial fermentation. In recent years, our increasing concerns on climate change and other environmental problems have been urging us to move away from fossil resource-dependent chemical processes and move towards more sustainable processes for the bio-based production of chemicals and materials from renewable resources [2].

In 2004 and 2010, the U.S. Department of Energy (DOE) reported SA as one of the five most promising bio-based platform chemicals. Fermentative production of SA would provide even more environmental benefits as one mole of CO_2 is fixed per one mole of SA during the fermentation. Recognizing its potential importance, extensive research has been carried out globally, which led to the development of several cost-effective processes for fermentative SA production from renewable resources. The cost for fermentative SA production is estimated to be \$0.55–1.10 per kg, which is competitive to that of petrochemical process. Several plants producing SA have been established by companies such as Bioamber, Myriant, Succinity, and Reverdia [3].

SA is currently used as surfactant, ion chelator, additive in agricultural and food, and in pharmaceutical industries. The demand of SA as a platform chemical is expected to rapidly increase to an anticipated-market size of >700 000 tons per year by 2020 [3]. A much bigger market of SA is expected as a precursor for numerous industrially valuable chemicals including adipic acid (a precursor for Nylon x,6), 1,4-butanediol (1,4-BDO; a precursor for polyesters and Spandex), tetrahydrofuran (THF; an important solvent and a precursor for poly[tetramethylene ether] glycol), N-methylpyrrolidone (NMP; an important solvent in chemical and lithium-ion battery industries), 2-pyrrolidone (a precursor for pharmaceuticals and vinylpyrrolidone), gamma-butyrolactone (GBL; a precursor for pesticides, herbicides, and pharmaceuticals), and other green solvents and chemicals. Furthermore, the use of SA can be extended to the synthesis of bio-based and/or biodegradable polymers such as polyesters: for example, polybutylene succinate (PBS) and polyamides (Nylon x,4) [4].

In this paper, we review the metabolic characteristics, metabolic engineering strategies, and fermentation performance indices of the most prominent SA producing microorganisms. In particular, metabolic engineering strategies employed for developing these SA producers to reduce the formation of byproducts as well as to maximize the yield and productivity of SA are revisited (Figure 1 and Table 1). Furthermore, the advantages and disadvantages of the prominent SA producing microorganisms are summarized in Table 2. Finally, perspectives

Figure 1



Metabolic pathways of *E. coli* [21,52] and *M. succiniciproducens* [32] and their best metabolic engineering strategies for the enhanced production of SA. Genes knocked out for the enhanced production of SA are marked with 'x'. For dual phase fermentation of *E. coli*, cells were first grown aerobically to a high concentration before SA production under anaerobic condition in the second phase. The *pyc* gene overexpressed in *E. coli* for dual phase fermentation is shown in bold arrow. The engineered *E. coli* strain KJ122 (Δ*ldhA*, Δ*adhE*, Δ*ackA*, Δ*focA-pflB*, Δ*mgsA*, Δ*poxB*, Δ*tdcDE*, Δ*citF*, Δ*aspC*, Δ*sfcA*) shown in the left produced 88 g/L of SA with the yield and productivity of 1.29 mol/mol glucose and 0.73 g/L/h, respectively, by anaerobic fed-batch fermentation. The engineered *E. coli* strain AFP111 (Δ*pfl*, Δ*ldhA*, Δ*ptsG*) overexpressing the *Rhizobium etli pyc* gene shown in the center produced 99.2 g/L of SA with the yield and productivity of 1.74 mol/mol glucose and 1.3 g/L/h, respectively; aerobic cell propagation stage was not taken into account for the calculation of SA yield and productivity. The engineered *M. succiniciproducens* PALFK (Δ*ldhA*, Δ*pta-ackA*, Δ*fruA)* shown in the right produced 78.41 g/L of SA with the yield and productivity of 1.64 mol/mol and 6.03 g/L/h, respectively, from sucrose and glycerol by anaerobic fed-batch fermentation. Genes shown are: *focA*, formate transporter; *mgsA*, methylglyoxal synthase; *poxB*, pyruvate dehydrogenase; *tdcDE*, propionate kinase/acetate kinase; *citF*, citrate lyase; *aspC*, aspartate aminotransferase; *pfl*, pyruvate formate lyase; *ldhA*, lactate dehydrogenase; *ptsG*, glucose-specific PTS enzyme; *pyc*; pyruvate carboxylase; *pta*, phosphate acetyltransferase; *ackA*, acetate kinase; *fruA*, fructose specific PTS system.

on further performance improvement and industrial-scale bio-based production of SA from renewable resources are discussed.

Production of succinic acid

SA, an intermediate of the tricarboxylic acid (TCA) cycle and one of the end products of anaerobic metabolism, is synthesized in almost all microbe, plants, and animal cells. Among these organisms, bacteria and fungi have been recognized as suitable hosts for the efficient production of SA. Much effort has been exerted to develop processes for the bio-based production of SA using several fungal/yeast strains such as *Aspergillus niger*, *Aspergillus fumigatus*, *Byssochlamys nivea*, *Candida tropicalis*, *Lentinus degener*, *Paecilomyces varioti*, *Penicillium viniferum*, *Saccharomyces cerevisiae*, and *Pichia kudriavzevii* (*Issatchenkia orientalis*). In the case of bacteria, the following strains have been employed for SA production: *Actinobacillus succinogenes*, *Anaerobiospirillum succiniciproducens*, *Corynebacterium glutamicum*, *Escherichia coli*, *Mannheimia succiniciproducens*, and *Basfia succiniciproducens* (very similar strain to *M. succiniciproducens*). Among these, this paper reviews the metabolic engineering strategies employed for developing succinic acid producers based on *S. cerevisiae*, *P. kudriavzevii*, *E. coli*, *M. succiniciproducens*, *B. succiniciproducens*, *A. succinogenes*, and *C. glutamicum*, as representative examples of those currently under commercialization. In comparing the results, those that employed dual phase fermentation (e.g., cell growth phase followed by SA production phase in the cases of *E. coli* and *C. glutamicum*) are indicated so that the true performance indices can be better understood.

Saccharomyces cerevisiae

S. cerevisiae is a well characterized eukaryotic microorganism that has been most widely used for industrial bioethanol production. Also, it has been employed as a platform strain for the production of various chemicals thanks to the availability of numerous genetic, metabolic engineering, and omics tools. *S. cerevisiae* does not normally produce SA as a fermentation end product. However, unlike bacterial succinic acid producers that prefer to grow at neutral pH, *S. cerevisiae* can grow within a wide pH range of 3–6, which offers a great advantage for SA production. The ability to grow at low pH reduces the need for neutralization to produce SA, and thus, generation of salts (e.g., gypsum) can