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Chemostat culture characterization of *Escherichia coli* mutant strains metabolically engineered for aerobic succinate production: A study of the modified metabolic network based on metabolite profile, enzyme activity, and gene expression profile

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Abstract

Various *Escherichia coli* mutant strains designed for succinate production under aerobic conditions were characterized in chemostat. The metabolite profiles, enzyme activities, and gene expression profiles were studied to better understand the metabolic network operating in these mutant strains. The most efficient succinate producing mutant strain HL27659k was able to achieve a succinate yield of 0.91 mol/mol glucose at a dilution rate of 0.1/h. This strain has the five following mutations: *sdhAB*, (*ackA-pta*), *poxB*, *iclR*, and *ptsG*. Four other strains involved in this study were HL2765k, HL276k, HL2761k, and HL51276k. Strain HL2765k has mutations in *sdhAB*, (*ackA-pta*), *poxB* and *iclR*, strain HL276k has mutations in *sdhAB*, (*ackA-pta*) and *poxB*, strain HL2761k has mutations in *sdhAB*, (*ackA-pta*), *poxB* and *icd*, and strain HL51276k has mutations in *iclR*, *icd*, *sdhAB*, (*ackA-pta*) and *poxB*. Enzyme activity data showed strain HL27659k has substantially higher citrate synthase and malate dehydrogenase activities than the other four strains. The data also showed that only *iclR* mutation strains exhibited isocitrate lyase and malate synthase activities. Gene expression profiles also complemented the studies of enzyme activity and metabolites from chemostat cultures. The results showed that the succinate synthesis pathways engineered in strain HL27659k were highly efficient, yielding succinate as the only major product produced under aerobic conditions. Strain HL27659k was the only strain without pyruvate accumulation, and its acetate production was the least among all the mutant strains examined.

Keywords: *Escherichia coli*; Metabolic engineering; Succinate production; Aerobic fermentation; Real-time RT-PCR; Chemostat; Enzyme activity

1. Introduction

The production of succinate has been an area of recent interest due to its value as a precursor to various commodity chemicals used in industries like food, pharmaceutical, detergent, and polymer (Zeikus, 1980; Zeikus et al., 1999). Metabolic engineering to enhance succinate production in bacteria has the potential to significantly improve the economics of the succinate market, especially when coupled with the use of renewable carbohydrates (Schilling, 1995). Examples include the high yield production of succinic acid from wood hydrolysate, whey, or glycerol by *Anaerobiospirillum succiniciproducens* through improvement of its fermentation conditions (Lee et al., 2000, 2001, 2003b; Samuelov et al., 1999). Other organisms that also have the innate capability for high yield succinic acid production include the facultative anaerobe

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Actinobacillus succinogenes (Van der Werf et al., 1997), and the organism from bovine rumen *Mannheimia succiniciproducens*, which had been shown to be able to produce succinic acid from whey and corn steep liquor (Lee et al., 2002, 2003a).

Various metabolic engineering strategies have been applied to improve succinate production in *Escherichia coli* (Chatterjee et al., 2001; Gokarn et al., 1998, 2000, 2001; Goldberg et al., 1983; Hong and Lee, 2001; Sánchez et al., 2005a, b; Stols and Donnelly, 1997; Vemuri et al., 2002a; Wang et al., 1998). Because *E. coli*, naturally, only produces succinate under anaerobic conditions in minimal quantities (Clark, 1989), numerous genetic modifications have been performed on *E. coli* to enhance succinate production. Genetic engineering coupled with optimization of production conditions has shown promising results for large-scale production of succinate from *E. coli*. This makes succinate production in *E. coli* competitive with that of other organisms like *A. succiniciproducens*. A genetically improved *E. coli* mutant strain (AFP111/pTrc99A-*pyc*) grown anaerobically in optimized fed batch conditions was shown to achieve succinate production of 99.2 g/L with yield of 110% and productivity of 1.3 g/L h (Vemuri et al., 2002b). Because of anaerobic fermentation process, disadvantages include poor biomass generation, slow carbon throughput, and, these slow product formation, *E. coli* was also genetically engineered to produce succinate under aerobic conditions (Lin et al., 2005a, b). Aerobic fed batch culture of a genetically modified *E. coli* mutant strain (HL27659k(pKK313)) was shown to produce 58.3 g/L of succinate with yield of 94% and productivity of 1.1 g/L h (Lin et al., 2005c).

In this study, several *E. coli* mutant strains constructed by Lin et al. during the development of aerobic succinate production systems were selected for characterization to further understand their metabolic functions, as a result of multiple pathway inactivations. This is important for understanding how the pathway manipulations in aerobic central metabolism affected the metabolic network and enabled aerobic succinate production in *E. coli*. These mutant strains were characterized in chemostat culture for their metabolite profiles, enzyme activities, and gene expression profiles. Enzyme activities and gene expression patterns were examined for those involved in pathways that affect the aerobic succinate production system. Combining gene expression profiles with enzyme activity and metabolite profiles provides a holistic approach for better understanding the connections between genes, proteins, and metabolites.

The mutations in the mutant strains were strategically created during the development process to enable

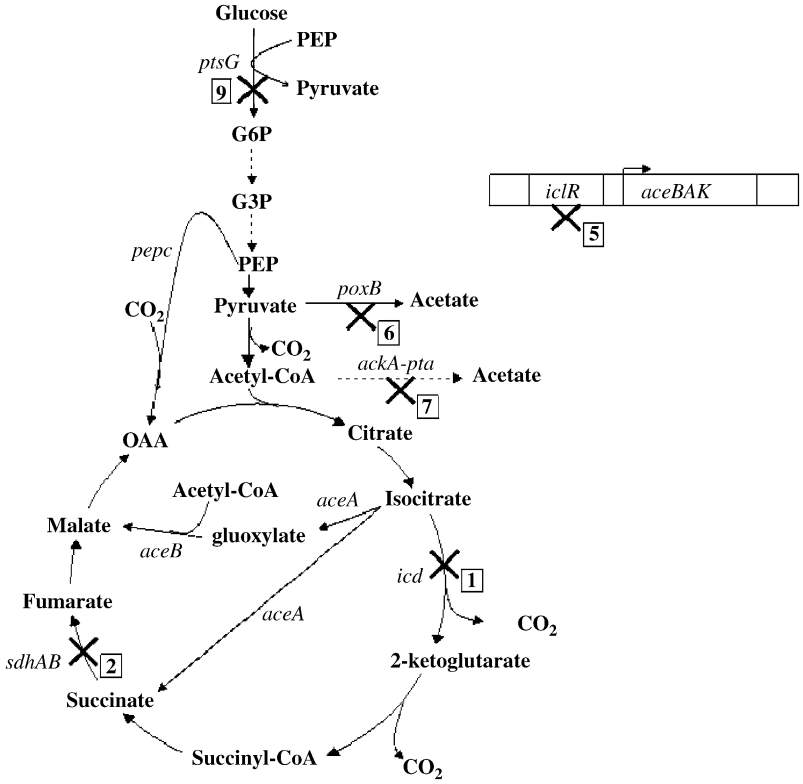

Fig. 1. Genetic engineering of glycolysis, TCA cycle, and glyoxylate bypass in the development of aerobic succinate production systems (Lin et al., 2005b). 1 is *icd* knockout, 2 is *sdhAB* knockout, 5 is *iclR* knockout, 6 is *poxB* knockout, 7 is *ackA-pta* knockout, and 9 is *ptsG* knockout.