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Design of homo-organic acid producing strains using multi-objective optimization

Tae Yong Kim [b,1], Jong Myoung Park [a,c,1], Hyun Uk Kim [a,c,e,1], Kwang Myung Cho [b,*], Sang Yup Lee [a,c,d,e,**]

[a] Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering (BK21 Plus Program), Center for Systems and Synthetic Biotechnology, Institute for the BioCentury, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Republic of Korea
[b] Biomaterials Laboratory, Material Research Center, Samsung Advanced Institute of Technology, Suwon 443-803, Republic of Korea
[c] BioInformatics Research Center, KAIST, Daejeon 305-701, Republic of Korea
[d] BioProcess Engineering Research Center, KAIST, Daejeon 305-701, Republic of Korea
[e] The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hørsholm, Denmark

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ABSTRACT

Production of homo-organic acids without byproducts is an important challenge in bioprocess engineering to minimize operation cost for separation processes. In this study, we used multi-objective optimization to design *Escherichia coli* strains with the goals of maximally producing target organic acids, while maintaining sufficiently high growth rate and minimizing the secretion of undesired byproducts. Homo-productions of acetic, lactic and succinic acids were targeted as examples. Engineered *E. coli* strains capable of producing homo-acetic and homo-lactic acids could be developed by taking this systems approach for the minimal identification of gene knockout targets. Also, failure to predict effective gene knockout targets for the homo-succinic acid production suggests that the multi-objective optimization is useful in assessing the suitability of a microorganism as a host strain for the production of a homo-organic acid. The systems metabolic engineering-based approach reported here should be applicable to the production of other industrially important organic acids.

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1. Introduction

Microbial strains have extensively been engineered to produce industrially valuable chemicals through biorefinery processes using renewable biomass as raw material (Zhuang et al., 2013). This requires systematic engineering of microbial metabolic network because cell's objective of maximum growth has a conflict with our objective of enhanced production of a target chemical (Kim et al., 2008a). Thanks to the hitherto accumulated knowledge on microbial physiology and high-throughput techniques, systems metabolic engineering has enabled efficient strain design that allows overproduction of various industrial chemicals (Kim et al., 2008b; Lee et al., 2012). In systems metabolic engineering, one of the useful strategies has been genome-scale metabolic modeling and simulation to predict effective gene manipulation targets at genome-scale (Lewis et al., 2012; Park et al., 2009). Recent successful examples of systems metabolic engineering with constraint-based flux analysis include production of 1,4-butanediol (Yim et al., 2011), daptomycin (Huang et al., 2012), malonyl-CoA (Xu et al., 2011), and putrescine (Park et al., 2012).

Prediction of gene manipulation targets using constraint-based flux analysis typically involves the use of multiple objective functions, including maximization of cellular growth rate and target chemical production rate (Burgard et al., 2003; Tepper and Shlomi, 2010; Yim et al., 2011). The rationale for the use of two or more objective functions is to increase the production of target chemicals while achieving acceptable levels of the other objectives, such as cell growth rate and minimal byproducts secretion. From the perspective of bioprocess engineering, however, gene manipulation targeting for the minimal production of byproducts has not been systematically investigated using constraint-based flux analysis although high operation costs associated with separation and purification of the target chemical from other byproducts are an important issue.

In general, 50–80% of the total processing costs for the bio-based organic acid production are caused by the downstream processes (Cukalovic and Stevens, 2008; Hermann and Patel,

* Corresponding author. Fax: +82 31 8061 1315.
** Correspondence to: Department of Chemical and Biomolecular Engineering, KAIST, Daejeon 305-701, Republic of Korea. Fax: +82 42 350 8800.
E-mail addresses: km3.cho@samsung.com (K.M. Cho), leesy@kaist.ac.kr (S.Y. Lee).
[1] These authors contributed equally to this work.

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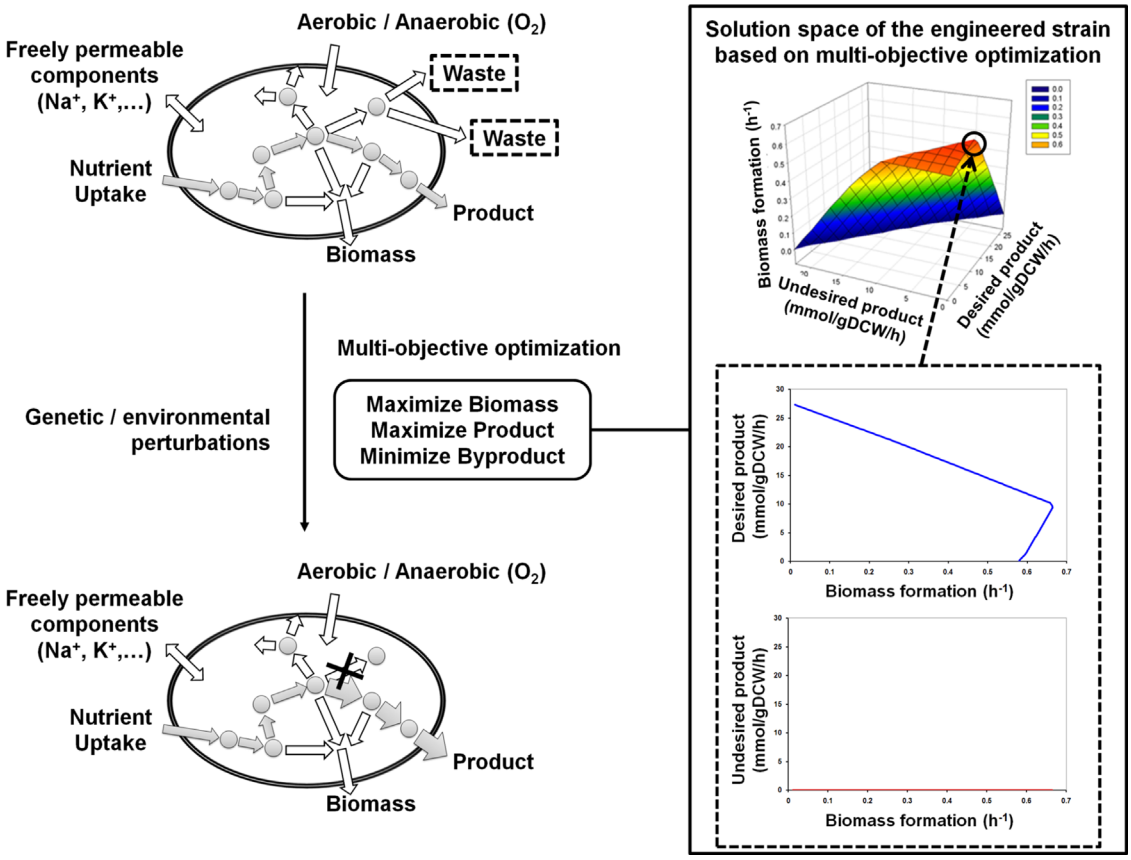

Fig. 1. Selection strategy of gene knockout targets for the homo-organic acid production using multi-objective optimization.

2007). Major challenges associated with the separation of organic acids are attributed to: the presence of target chemicals in a low concentration in the fermentation broth; the presence of various byproducts; and the necessity to remove salts from target chemicals, caused by neutralization (e.g., addition of ammonium) to control pH during fermentation (Song and Lee, 2006; Yuzbashev et al., 2011). For instance, production of succinic acid by several different microorganisms suggests that acetic acid is one of the major byproducts, requiring the costly separation process (Agarwal et al., 2005; Song and Lee, 2006; Zeikus et al., 1999). Such challenges in the downstream process need to be overcome for successful industrial production of organic acids by microbial fermentation.

Because of the issue associated with separation and purification processes, production of homo-organic acids without byproducts has been an important challenge in systems metabolic engineering. In the past, gene manipulations for this purpose have been intuitively conducted based on microbial physiological characteristics. Representative examples of microbial production of homo-organic acids include acetic (Causey et al., 2003), lactic (Zhu et al., 2007) and succinic acids (Jantama et al., 2008) using *Escherichia coli*. Homo-production of these organic acids required extensive gene manipulations, engineering more than 5–10 genes/operons. Examination of these strains producing homo-organic acids shows that it should be possible to achieve sufficiently high production titer of homo-organic acid with fewer gene manipulations, compared to the approach involving intuitively selected gene targets. This is important for the metabolic stability of microbial host strain and reduced experimental effort and costs.

To this end, we attempted to facilitate a downstream process by efficiently minimizing the secretion of byproducts during strain development stage using the multi-objective optimization. Specifically, we utilized constraint-based flux analysis with multiple objective functions to design *E. coli* strains with a goal of maximally increasing the production of target organic acids, while maintaining sufficiently high growth rate and minimizing undesired byproducts (Fig. 1). This systems approach was applied to the homo-production of acetic, lactic and succinic acids as demonstrations. In particular, productions of homo-acetic and homo-lactic acids were experimentally verified by constructing *E. coli* strains with minimal gene knockouts. The resulting mutant strains should be able to serve as competitive base strains for further metabolic engineering if needed. Prediction of gene targets for homo-succinic acid production by *E. coli* was not successful, indicating that *E. coli* is not the best host strain for homo-succinic acid production. This study demonstrates the usefulness of overall bioprocess optimization by the multi-objective optimization of a genome-scale metabolic model.

2. Materials and methods

2.1. Prediction of gene knockout targets using the multi-objective optimization for the homo-organic acid production

Previously reported genome-scale metabolic network models of *E. coli* (Lee et al., 2005b; Reed et al., 2003) and a succinic acid-overproducing bacterium, *Mannheimia succiniciproducens* (Kim et al., 2007), were used in simulating their metabolism under various genotypic and environmental conditions. The metabolic models are mathematically described in a stoichiometric matrix for each biochemical reaction and its associated metabolites, while each metabolite is mass-balanced (Orth et al., 2010). Because the genome-scale

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