Rewiring Metabolism to Construct a Yeast Strain Capable of Producing 2,3-butanediol Without Ethanol and Glycerol Production

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Project Goals

Complete use of glucose and xylose is a prerequisite for producing biofuels and chemicals from lignocellulosic biomass. We have constructed an engineered *Saccharomyces cerevisiae* strain (CT2) capable of rapidly and efficiently producing ethanol from glucose and xylose. To construct a platform yeast strain for the production of CABBI target molecules (organic acids, alcohols, and lipids), metabolic designs enabling the maximum production of target molecules with no byproducts were tested in the CT2 strain using 2,3-butanediol (2,3-BDO) as a target molecule. We envision that this new strain, which efficiently and rapidly produces 2,3-BDO without byproduct formation, can be further engineered to produce other CABBI target molecules via modifications in metabolic, redox balance, and energetic pathways.

Abstract

2,3-butanediol (2,3-BDO) is a versatile commodity chemical which can be used for making synthetic rubbers, anti-freeze, and liquid fuel additives. While production of 2,3-BDO by microbial fermentation has been demonstrated before, use of potential human pathogenic microorganisms, formation of byproducts, and low productivities limited the commercialization of the fermentation processes.

As an alternative, we engineered *Saccharomyces cerevisiae*, which is a GRAS (generally recognized as safe) and preferred microorganism by industrial biotechnology companies, and achieved efficient and rapid production of 2,3-BDO without byproduct formation. Key objectives were to 1) introduce a 2,3-BDO production pathway, 2) to eliminate the formation of byproducts, such as ethanol and glycerol, and 3) to re-wire redox-balancing metabolic pathways for the production of 2,3-BDO with a high yield (>80%) and productivity (>1 g/L·h).

To these ends, we first deleted major isozymes of *PDC* (pyruvate dehydrogenase) and *ADH* (acetaldehyde dehydrogenase) genes in *S. cerevisiae* to minimize ethanol production while maintaining sufficient acetyl-CoA pool for cell growth. Second, heterologous genes—*alsS,* coding for acetolactate synthase, and *alsD,* coding for acetolactate decarboxylase, from *B. subtilis* were overexpressed. Third, we eliminated glycerol accumulation by deleting both *GPD1* and *GPD2*, which code for glyceraldehyde-3-phosphate dehydrogenase. Fourth, we introduced a NAD^+ regenerating pyruvate-malate cycle to resolve the redox imbalance from deletion of the glycerol

producing pathway. Lastly, we enhanced the expression level of *PYC1* and *PYC2* to enhance the NAD^+ regenerating capability of the pyruvate-malate cycle. As a result, our best strain was able to produce 2,3-BDO with a much higher productivity (1.1 g/L·h) than previously constructed 2,3-BDO producing strains (0.1~0.2 g/L·h) in a batch fermentation with 100 g/L of glucose. In addition to the rapid production of 2,3-BDO, the best strain produced negligible amounts of glycerol and ethanol. As such, the engineered yeast offers the potential for economical downstream processing and efficient catalytic upgrading of 2,3-BDO.

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