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Analyzing redox balance in a synthetic yeast platform to improve utilization of brown macroalgae as feedstock

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ABSTRACT

Macroalgae have high potential to be an efficient, and sustainable feedstock for the production of biofuels and other more valuable chemicals. Attempts have been made to enable the co-fermentation of alginate and mannitol by *Saccharomyces cerevisiae* to unlock the full potential of this marine biomass. However, the efficient use of the sugars derived from macroalgae depends on the equilibrium of cofactors derived from the alginate and mannitol catabolic pathways. There are a number of strong metabolic limitations that have to be tackled before this bioconversion can be carried out efficiently by engineered yeast cells.

An analysis of the redox balance during ethanol fermentation from alginate and mannitol by *Saccharomyces cerevisiae* using metabolic engineering tools was carried out. To represent the strain designed for conversion of macroalgae carbohydrates to ethanol, a context-specific model was derived from the available yeast genome-scale metabolic reconstructions. Flux balance analysis and dynamic simulations were used to determine the flux distributions. The model indicates that ethanol production is determined by the activity of 4-deoxy-l-erythro-5-hexoseulose uronate (DEHU) reductase (DehR) and its preferences for NADH or NADPH which influences strongly the flow of cellular resources. Different scenarios were explored to determine the equilibrium between NAD(H) and NADP(H) that will lead to increased ethanol yields on mannitol and DEHU under anaerobic conditions. When rates of mannitol dehydrogenase and $DehR_{NADH}$ tend to be close to a ratio in the range 1–1.6, high growth rates and ethanol yields were predicted. The analysis shows a number of metabolic limitations that are not easily identified through experimental procedures such as quantifying the impact of the cofactor preference by DEHU reductase in the system, the low flux into the alginate catabolic pathway, and a detailed analysis of the redox balance. These results show that production of ethanol and other chemicals can be optimized if a redox balance is achieved. A possible methodology to achieve this balance is presented. This paper shows how metabolic engineering tools are essential to comprehend and overcome this limitation.
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1. Introduction

Depletion of fossil resources, increasing demand for fuel and climate change have encouraged the use of more efficient and sustainable sources to produce valuable products and energy (Jang et al., 2012). Microbial fermentation of biomass from diverse sources has been used to overcome this challenge. Corn and sugarcane biomass have been successfully used to produce biofuels at high yields using well-established fermentation technology, but their long-term use is questionable due to the competition between fuel and food resources. Although lignocellulosic plant materials are an alternative, the process and related costs to release sugars are extremely high and complex. In the past years, macroalgae, so-called seaweeds, have attracted attention for their high potential as feedstock to produce sustainable biofuels and commodity chemical compounds. Brown macroalgae has several key features: (1) its cultivation does not impact food supplies since it does not require fresh water resources or arable land; (2) brown macroalgae do not contain lignin which implies that its cell wall is structurally flexible; and (3) macroalgae are already being mass-cultivated in several countries (Jung et al., 2013).

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The major polysaccharide constituent of brown macroalgae is alginate (30–60% of the total carbohydrates). Alginate is composed of β-D-mannuronate (M) and α-L-guluronate (G) which are linked by 1,4-glycosidic bonds. These uronic acids can be arranged as M-blocks, G-blocks and alternative blocks of M and G units (Rehm, 2009). Mannitol and glucan (present as laminarin and cellulose) complete the carbohydrate composition. A key criterion for the economic and efficient use of the various sugars derived from brown macroalgae implies identification or design of microorganisms that can metabolize these carbohydrates. Metabolic engineering and genetic transformation play an important role to improve product yields and in the efficient use of this biomass. At present, many efforts are being made to engineer well-characterized microorganisms to utilize alginate and mannitol as carbon sources (Enquist-Newman et al., 2014; Wargacki et al., 2012). Enquist-Newman et al. (2014) have shown that ethanol can be produced from the co-fermentation of mannitol and an alginate monomer (4-deoxy-L-erythro-5-hexoseulose uronate, or DEHU) by *Saccharomyces cerevisiae*. They circumvent the limitations of the native strain by engineering both the mannitol and alginate catabolic pathways (Enquist-Newman et al., 2014). The native mannitol metabolic pathway was deregulated. The *Vibrio splendidus* alginate metabolism pathway was reconstructed in yeast together with the integration of the DEHU transporter of *Asteromyces cruciatis*. The efficiency of the mannitol and alginate metabolism pathways to produce ethanol depends on the redox control. Mannitol as a polyol generates excess reducing equivalents which must be redox balanced through an electron shunt. For ethanol production, alginate metabolism provides a counter balance to consume two reducing equivalents per mole of alginate. This electron transfer enabled ethanol fermentation from these sugars. Thus, a key step in this specific design was the selection of a DEHU reductase (DehR) with optimal cofactor preference for redox-balance, it preferentially uses NADH and co-uses NADH and NADPH. Fig. 1 describes the cofactor balance to generate ethanol in the engineered *S. cerevisiae*.

Ethanol fermentation from mannitol and DEHU was achieved under two specific growth conditions, 1:2 M ratio of DEHU:mannitol at 6.5% (w/v) and 9.8% (w/v) total sugars where the ratios of mannitol:DEHU consumptions were 2.4 and 2.1, respectively. In both cases, glycerol is the main by-product as it helps to achieve a cofactor balance. However, no other ratios of mannitol:DEHU uptakes were reported. In addition, metabolism of DEHU could lead to a deficit of NADH. Yeast needs an excess of NADH to generate ethanol. In order to increase the bioconversion to ethanol from brown macroalgae sugars and allow biomass formation and metabolic maintenance, it is necessary to quantify the ability of DehR for co-use of NADH and NADPH and its impact on the redox balance and ethanol production under different ratio consumption rates. In this study, we aim to identify the optimal flux distribution through DehR to decrease by-products and increase the ethanol yield on alginate and mannitol. To represent the engineered strain used for bioconversion of brown macroalgae sugars to ethanol, we used a context-specific model derived from the most updated yeast genome-scale metabolic reconstruction (Heavner et al., 2012). We evaluated the network by comparing in silico biomass formation and by-production rates to *in vivo* measurements. Flux balance analysis was used for *in silico* characterization of the current metabolic state of the yeast platform and the strategies proposed to achieve the optimal distribution (Orth et al., 2010).

2. Methodology

2.1. Metabolic model

To mimic the cellular behaviour of the synthetic yeast platform for bioconversion of brown macroalgae sugars to ethanol a previously described genome-scale model was used to represent *S. cerevisiae* metabolism. This corresponds to an updated version of Yeast5 (Heavner et al., 2012). The yeast systems biology community carried out this reconstruction and it includes 910 genes, 3490 reactions and 2220 metabolites. The network is fully compartmentalized, elementally-balanced and no regulatory constraints have been included yet. The reconstruction is available at http://www.comp-sys-bio.org/yeastnet/.

The biochemical reactions of the reconstruction define a stoichiometric matrix that allows testing of the capabilities of the system based on structural knowledge of the metabolic reaction network and steady-state flux distributions. The stoichiometry-

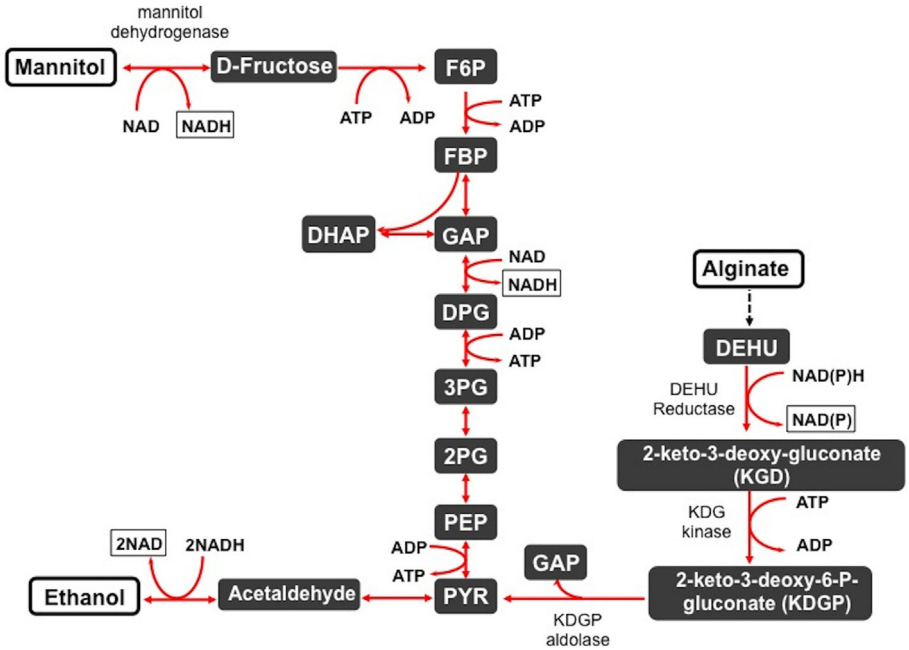

Fig. 1. Overview of cofactor requirements in the engineered *S. cerevisiae* strain.

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