Tolerant yeast *in situ* detoxifies major class of toxic chemicals while producing ethanol

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ABSTRACT

Renewable lignocellulosic materials contain abundant sugar source and biofuels conversion including cellulosic ethanol production from lignocellulosic biomass provides a sustainable alternative energy resource for a cleaner environment. In order to release the biomass sugars from the complex cellulosic structure for efficient microbial utilization, a pretreatment of lignocellulosic biomass is required. Pretreatment of lignocellulosic biomass, such as commonly used dilute acid hydrolysis, generates numerous toxic chemical compounds that inhibit microbial growth and subsequent fermentation. Overcoming major class of toxic chemicals is one of significant challenges for economic production of advanced biofuels including cellulosic ethanol. Scientists at ARS created the first tolerant industrial yeast strain *Saccharomyces cerevisiae* NRRL Y-50049 that is able to *in situ* detoxify major class of toxic chemicals, such as toxic aldehydes represented by furfural and 5-hydroxymethylfurfural (HMF), derived from lignocellulosic biomass pretreatment while producing ethanol. Using this tolerant industrial ethanologenic yeast strain, we defined the mode of action for reduction of furan aldehydes. Applying genomic technology, we revealed reprogrammed glycolysis and pentose phosphate pathways for the tolerant yeast in response to challenges of the toxic chemicals; identified key regulatory elements and candidate genes for the yeast tolerance; characterized genome expression and tolerant signaling pathways; detected global rewired networks, pathway interactions, and at least 44 downstream pathways involved in the yeast tolerance. Additionally, we defined a novel gene of aldehyde reductase *ARI1* in *S. cerevisiae* and later proposed a new gene family of aldehyde reductase containing at least four members in the yeast. We also found new gene functions of previously reported genes that involved in the detoxification and concluded that multiple gene-mediated NAD(P)H-dependent aldehyde reduction is a mechanism of *in situ* detoxification of furfural and HMF by the tolerant yeast. *S. cerevisiae* NRRL Y-50049 is a US patented strain and a valuable resource as a candidate for the next generation biocatalyst development for advanced biofuels production.

Keywords: advanced biofuels, in situ detoxification, next-generation biocatalyst, stress tolerance, yeast

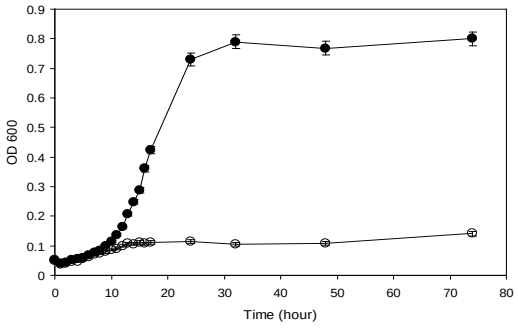

Figure 1. Comparison of cell growth for Saccharomyces cerevisiae NRRL Y-12632 (open circle) and strain Y-50049 (filled circle) in response to furfural and HMF at 12 mM each on a defined medium over time showing near normal growth of Y-50049 as an initial culture while Y-12632 failed to establish a culture under the inhibitor stress conditions.

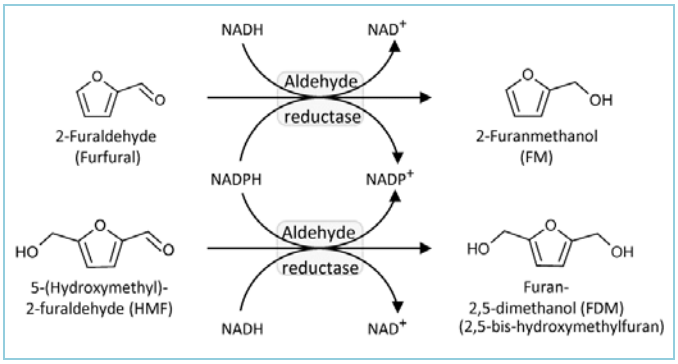

Figure 2. Conversion pathways of 2-furaldehyde (furfural) and 5-(hydroxymethyl)-2-furaldehyde (HMF) into 2-furanmethanol (FM) and furan-2,5-dimethanol (FDM) coupled with NADH and/or NADPH and catalyzed by multiple reductases.

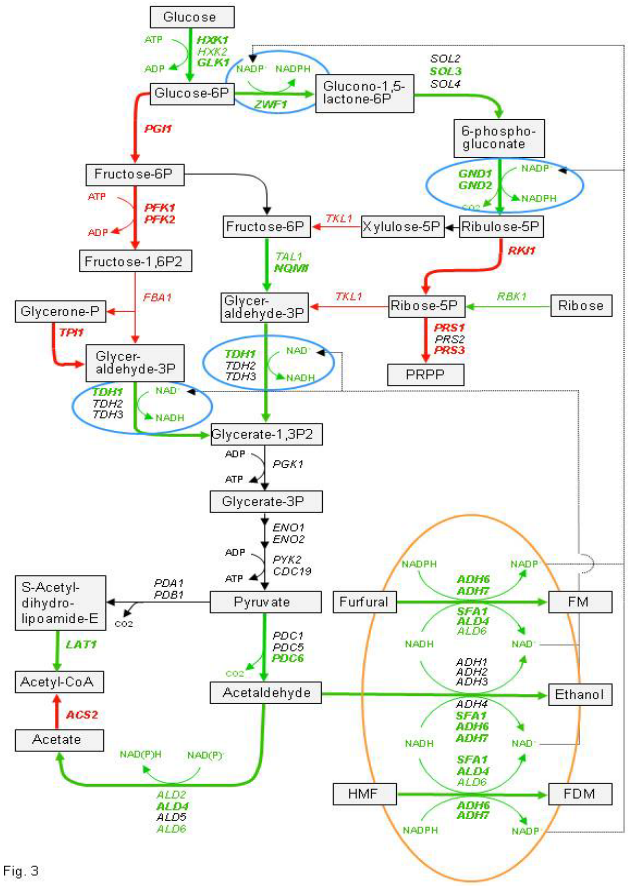

Fig. 3

Figure 3. A schematic illustration of glucose metabolic pathways and conversion of furfural and HMF by tolerant Saccharomyces cerevisiae NRRL Y-50049 inferred by metabolic profiling analysis and quantitative mRNA expression analysis compared with a wild type strain NRRL Y-12632. Black arrowed lines and letters indicate normal or near normal levels of reactions, expressions or pathways, green indicates enhanced, and red for repressed expressions, reactions, or pathways. Bolded lines and letters indicate the levels of expression and pathways are statistically significant. Key steps of enhanced NAD(P)H regenerations are circled in blue and significant aldehyde reductions circled in orange.

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