A SYNTHETIC REGULON ENHANCES THE FITNESS OF YEAST ON NON-NATIVE NUTRIENTS

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Metabolic engineering has enabled production of bio-based chemicals in organisms, usually by overexpressing the genes (heterologous or native genes) involved in the biochemical pathway to debottleneck rate limiting steps. Recent studies have shown that engineered regulatory systems, such as removing feedback inhibition, can further improve the performance of engineered strains. We hypothesize that engineering a global regulatory system (regulon) could provide a new paradigm in engineering biological systems and complement current tools available for metabolic engineering. To demonstrate this, we use the assimilation of xylose by *S. cerevisiae* as a test case. Xylose is a non-native sugar to this yeast, but an abundant natural sugar.

Currently, engineering xylose assimilation for biomass or ethanol production in *S. cerevisiae* has been limited to overexpression of initial genes in the pathway to convert xylose to xylulose-5-phosphate followed by expression of non-oxidative Pentose Phosphate Pathway genes to increase the flux towards glycolysis. However, growth involves coordinated control of multiple pathways involving carbon metabolism, cofactor regeneration, amino acid synthesis, nucleotide synthesis, cell cycle maintenance etc., and debottlenecking rate-limiting reactions in all of the necessary pathways required for growth in xylose would involve extensive pathway engineering. To get around this, we hypothesized that further enhancement in xylose utilization can be made by addressing the issue from a regulatory perspective rather than metabolic. To that end, we decided on a regulon engineering strategy whereby a sugar sensing regulon can be engineered to trigger transcriptional machinery when xylose is encountered thereby enhancing the growth and biocatalytic fitness in this non-native sugar.

Previous studies have shown that presence of xylose weakly upregulates galactose catabolic genes (GAL). This suggested that xylose can mimic galactose as an agonist of the GAL regulon, and that this system could serve as a platform to develop a xylose-dependent regulatory system. We first engineered the signal transduction step that increases the sensitivity and response kinetics of the GAL regulon for xylose as well as its native ligand, galactose. We further show that by switching ON the regulon using a dual positive feedback loop system we could achieve growth rates comparable to current evolutionary engineered strains. In this process, we also enhanced the galactose sensing capabilities of the sensor, thereby achieving higher growth rates on galactose than the wild type strains. Finally, we also show that under non-inducing conditions, strains carrying the xylose regulon show better growth fitness than strains with constitutive expression of xylose metabolic genes. Further increase in growth rates, xylose uptake, and specific chemical (including biofuels) production can be achieved by expanding the genes under the synthetic xylose regulon.

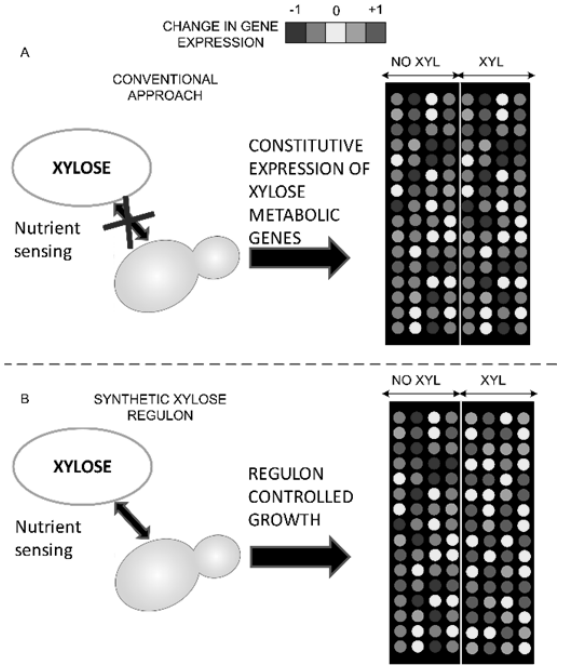

Figure 1: Schematic of xylose sensing and metabolic system in S. cerevisiae. (A) Conventional approaches to xylose metabolic engineering do not enable S. cerevisiae to sense xylose as a nutrient, resulting in pathways required for growth not completely turned ON and remain in the same state as when xylose is not present. (B) Our approach of regulatory engineering aims at activating the GAL regulon through xylose, resulting in turning ON of the necessary pathways for growth.