

Directed Evolution of Novel Yeast Species

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Objective: Progress towards converting from a geology-based to a biology-based economy depends critically on improving the efficiency and flexibility of biomass conversion through improvements in both thermochemical and biochemical processes. Improvements in the latter will benefit greatly from development of novel, adaptively evolved, hybrid yeast strains capable of aggressively fermenting sugars at elevated temperatures and ethanol concentrations from pretreated forest and agricultural residuals.

Background: For inexpensive, large-scale bioethanol production, producers will need to develop microbial systems that can ferment biomass from feedstocks such as corn stover, corn fiber, rice straw, cereal straw, bagasse, forestry waste products, and possibly switch grass (NREL, 2002). *Saccharomyces cerevisiae* is the organism of choice for the development of such a system. While *S. cerevisiae* is able to ferment the hexose sugars in cellulose to ethanol, it normally lacks the ability to produce ethanol by the fermentation of pentose sugars, xylose and L-arabinose, present in hemicellulose. Although it cannot utilize xylose as a sole carbon source, *S. cerevisiae* can catabolize this substrate, albeit slowly (van Zyl *et al.*, 1989), as it possesses the genes that encode the key steps in xylose metabolism. *S. cerevisiae* also has an endogenous xylulose kinase which converts xylulose to a compound that can enter a pathway eventually allowing for energy production and growth, and the evolution of CO₂ and ethanol (Ho 1989, Batt 1986). Wild-type *S. cerevisiae* contains all the necessary enzymes to allow growth on xylose as a sole carbon source.

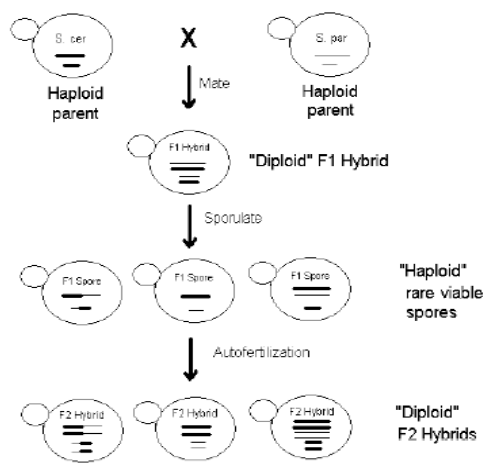


Figure 1: Process to create hybrid yeast species

Approach: Novel yeast species that can efficiently convert xylose from plant biomass to ethanol at elevated temperatures and ethanol concentrations will be generated through natural selection. The steps below describe the research methods and goals:

1: Create libraries of hybrid yeast strains which present large pools of standing genetic variation. We will use a method of creating hybrid yeast species developed by Greig *et al.*, depicted in Figure 1. We will isolate rare viable spores from the hybrid, which rapidly autofertilize to form hybrids. These will serve as our initial library stock for subsequent experiments, and will be frozen in glycerol. For our first round of evolutionary selections, we will either use cells directly from this stock, or cells from this stock which have been mutagenized to increase genetic variability. Thus all our initial experiments will use a genetically diverse pool of yeast hybrids.

2: Select, through the application of evolutionary pressures, hybrid yeasts exhibiting phenotypes useful for the production of ethanol from plant biomass. We will use chemostats to select for desirable traits (thermotolerance, ability to grow at high concentrations of ethanol, and the ability to grow on xylose) using the large pool of genetically variable, hybrid F2 yeast to inoculate chemostat cultures. These hybrid pools will be propagated vegetatively in continuous chemostat culture for ca. 320 generations, initially under selection for one phenotype. Having evolved phenotypes individually, we will mate the evolved strains with each other in all pairwise combinations (*e.g.*, xylose fermenters with high-temperature glucose fermenters). From each of these new hybrids, we will again select for rare viable spores, and use these to inoculate a second round of chemostat cultures, simultaneously selecting for two of the desired traits. Finally, we will mate each hybrid possessing two desired traits with a hybrid containing the remaining trait, and from each of these new strains we will again generate.

3: Design and test DNA microarrays that contain probes for *S. cerevisiae* and one of the other *Saccharomyces sensu stricto* strains to study genomic rearrangements and gene expression changes in evolved hybrid yeasts. We will wish to assay transcription in the hybrid strain with respect to that of the original parental strain, to understand the changes that have occurred to the levels of various enzymes involved in metabolism. In addition, we will also assay changes in genome architecture using microarrays and will look for recurrent alterations from different spores from a given hybrid, as well as across all hybrids. Identification of recurrent changes occurring while under selective pressure will be strongly indicative of restrictions to the possible evolutionary trajectories.

4: Perform a detailed comprehensive characterization of chromosomal rearrangements and gene expression changes that occur and characterize their stability. Because we will have monitored both the generation time of the cells and the frequency of neutral markers at each point in the experiment, we will know with a great degree of accuracy when each adaptive sweep has occurred. Thus, we will be able to retroactively examine both the DNA and RNA content of a cell population immediately prior to and following an adaptive sweep. These studies will yield fundamental insight into the changes in genome architecture and global transcription that underlie evolutionary adaptation to xylose fermentation, and growth under conditions of elevated ethanol concentration and high temperature. After we have selected strains with all three desirable traits, we will use microarrays to characterize their phenotypic (*i.e.*, transcriptional) and

karyotypic stability. There are at least two circumstances in an industrial setting where it is important for these strains to be stable. First, strains will need to be propagated under standard laboratory conditions in order to produce large populations for inocula. Typically this would be carried out as a short-term batch culture. Second, strains must be stable over the course of prolonged industrial fermentations. Thus, we will propagate evolved strains in batch cultures for a short time (10 generations) under standard laboratory culture conditions medium at 30°C), as well as in extended (50 generations) fed-batch cultures that mimic industrial fermentations.

References:

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