

Towards Cellulosic Ethanol : Identification of Naturally Occurring Yeast Strains with a Single Gene Trait for Xylose Utilization

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Abstract

The US Department of Energy has identified the need to supplement transportation fuel supplies with sustainable, domestic resources as one of the nation's highest energy priorities. Progress towards converting from a geology-based to a biology-based energy economy depends critically on improving the efficiency and flexibility of biomass conversion through improvements in both thermochemical and biochemical processes. Improvements in the latter require development of a microbial system capable of aggressively fermenting at elevated temperatures and ethanol concentrations the mixture of five- and six-carbon sugars that result from thermochemical and enzymatic pre-treatment. In order to utilize all of the substrate found in these cellulosic and hemicellulosic feedstocks it will be necessary to develop a microbial system can ferment both hexose and pentose sugars.

Saccharomyces cerevisiae, because of its GRAS ("Generally Recognized As Safe") and model organism status, is the organism of choice for the development of such a system. While *S. cerevisiae* is able to ferment hexose sugars to ethanol, it normally lacks the ability to produce ethanol by the fermentation of xylose, the predominant pentose sugar present in hemicellulose. On the other hand, although it cannot utilize xylose as a sole carbon source, *S. cerevisiae* can catabolize this substrate, albeit slowly, as it possesses the genes that encode xylose reductase, xylitol dehydrogenase, and xylulokinase, the key steps in xylose metabolism.

We propose to use evolutionary engineering techniques to select improved strains of yeast capable of fermenting xylose. As a first step towards this end, we have carried out a screen of our strain collection in an effort to identify naturally occurring strains that have any ability to utilize xylose, either for biomass accumulation, or growth and division. Here we report the identification of a number of yeast wine strains, all of which harbor a single locus that segregates in Mendelian fashion that confers a modest xylose positive phenotype.

Tiling microarray analysis probably localizes the gene to chr.15R

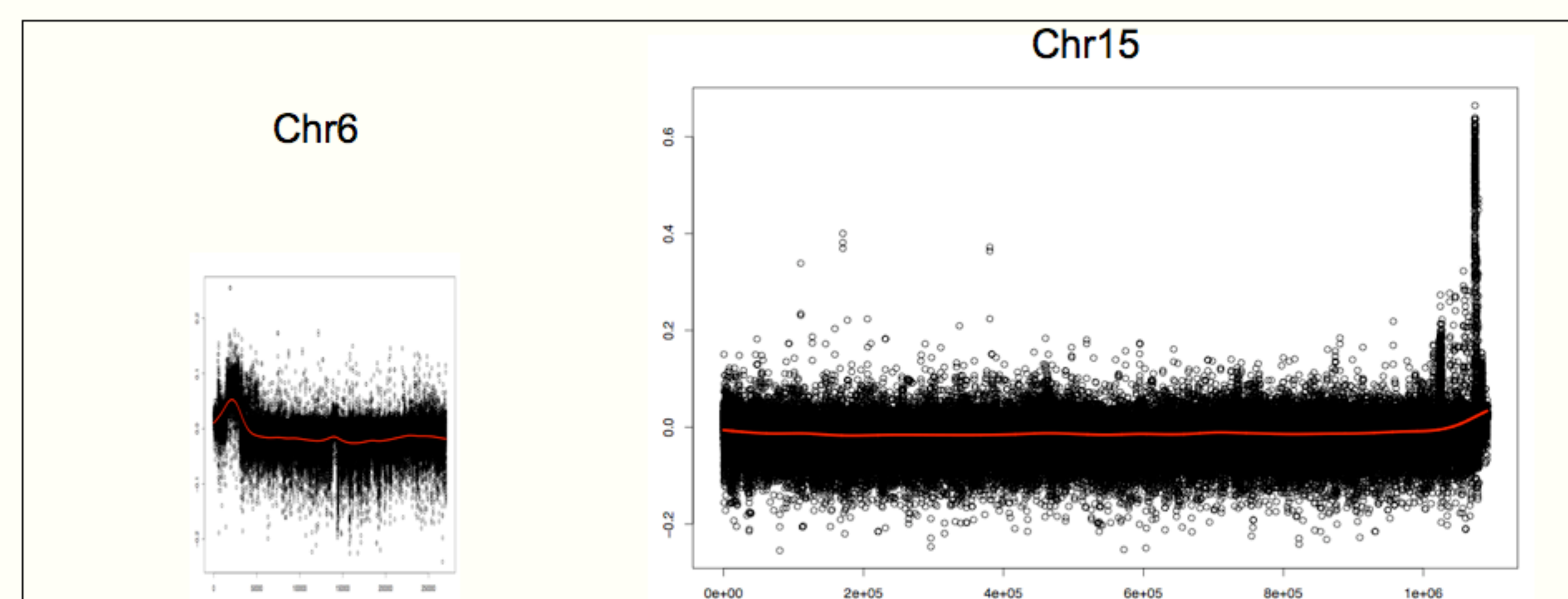


Figure 5. Pools of DNA from 39 xylose positive and 39 xylose negative segregants from a cross to a xylose negative laboratory strain were hybridized to Affymetrix tiling microarrays. The log ratio between the pools strongly suggests that the gene of interest localizes to the right arm of chromosome 15 (right), though there are additional peaks of lower significance, e.g. on the left arm of chromosome 6 (left).

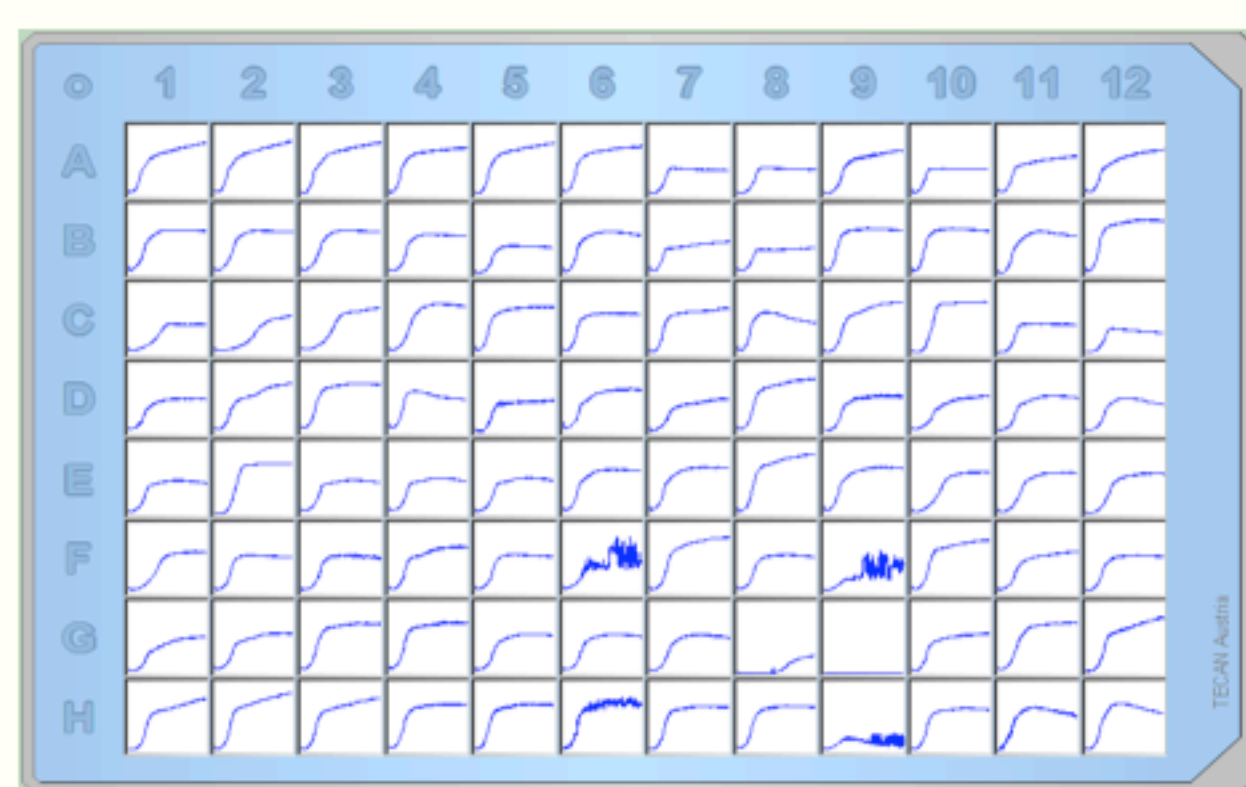


Figure 1: Typical results from initial screen for growth in YP plus 2% xylose. Putative positives (e.g. A1 thru A6) show a second phase of growth, after the trehalose in the YP has been exhausted.

We tested 430 different strains (900 collection entries):

173 wine *S.cerevisiae*,
82 non-wine *S.cerevisiae*,
5 ale yeasts
(260 *S.cerevisiae* total)

13 *S. paradoxus*
3 *S. kudriavzevii*
8 *S. bayanus* or *uvarum*
2 *S. cariocanus*
2 *S. mikatae*
7 *S. bayanus+uvarum*
48 *S. bayanus+cerevisiae*
(including *S. pastorianus*)
28 viable spores from above
11 *S. paradoxus+cerevisiae*
48 viable spores from above

The Xylose phenotype is linked to a gene on chromosome 15

	G418	Xylose
2 A	+	-
B	-	+
C	+	-
D	-	+
10A	+	-
B	-	+
C	+	-
D	-	+

To verify location of the gene as being on chromosome 15, we crossed wine strain derivatives to strains with marked deletions either on the left arm of chromosome 6 (*yfl054c::kanR*) or the right arm of chromosome 15 (*phr1::kanR*). Six complete tetrads from both crosses were evaluated for growth in xylose. Xylose positive phenotype was unlinked to deletion in *YFL054C*, while always segregated away from deletion in *PHR1* (two example tetrad patterns shown to the left).

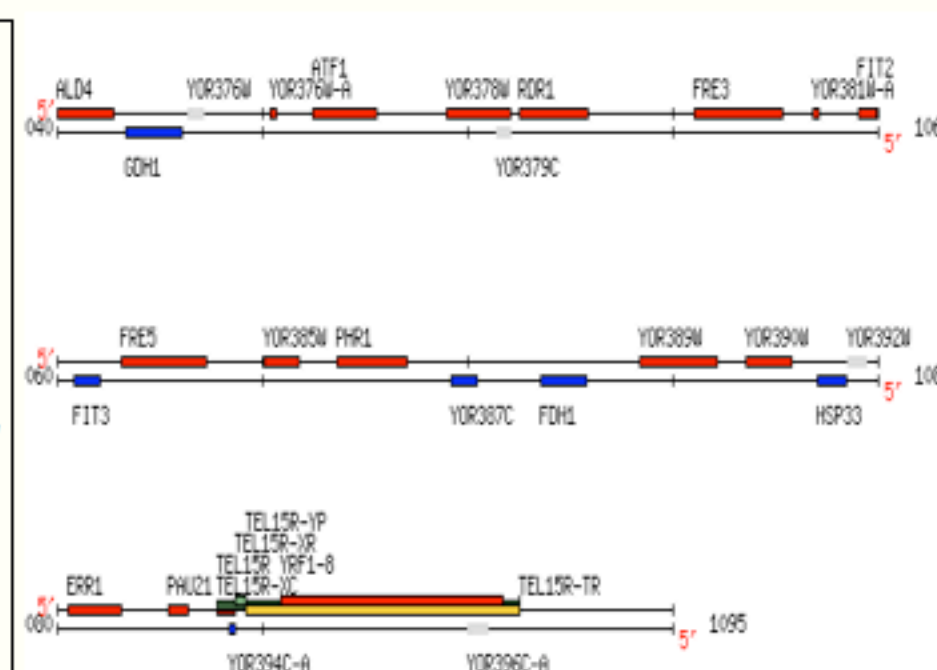


Figure 6: Annotated Features in the vicinity of Chromosome 15R. The large peak shown in Figure 5 maps to *FDH1*, while a smaller adjacent peak maps to *YOR389W*, and just downstream of *HSP33*.

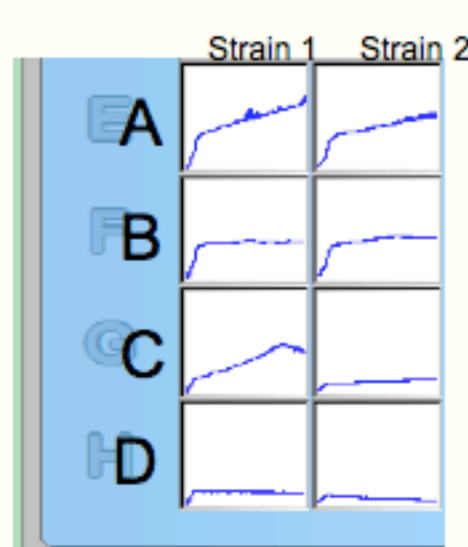
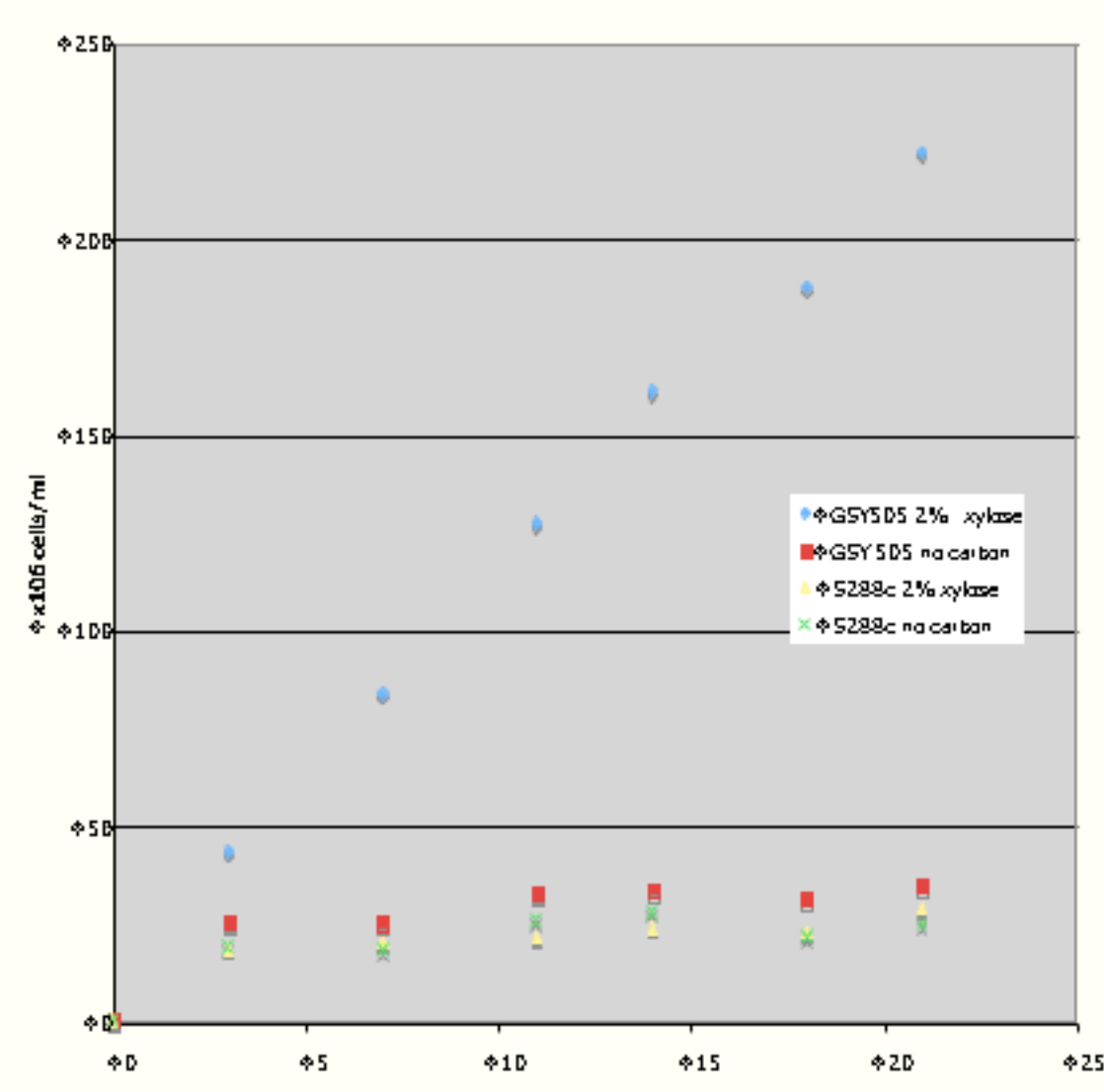


Figure 2: Putative positive strains from the first screen were retested comparing growth pattern in rich medium with xylose (A) or without carbon source (B), and in minimal medium with Xylose (C) or without carbon source (D). If the OD600 increased in the presence of xylose, but not in its absence, then the strain was considered able to utilize xylose. In the figure to the left, strain 1 shows growth in minimal medium with xylose, but not without xylose, whereas strain 2 does not.

Figure 3: The triple hybrid, GSY505 is able to double many times using xylose as its sole carbon source.



Xylose Positives:

- 21 *S.cerevisiae* wine strains and strains collected from vineyards.
- 7 *S. cerevisiae* + *S.bayanus* hybrids.
- 1 *S. uvarum* + *S. bayanus* + *S. cerevisiae* hybrid.
- 2 *S.uvarum* + *S. bayanus* hybrids.

The Xylose positive phenotype in wine strains is encoded by a single locus

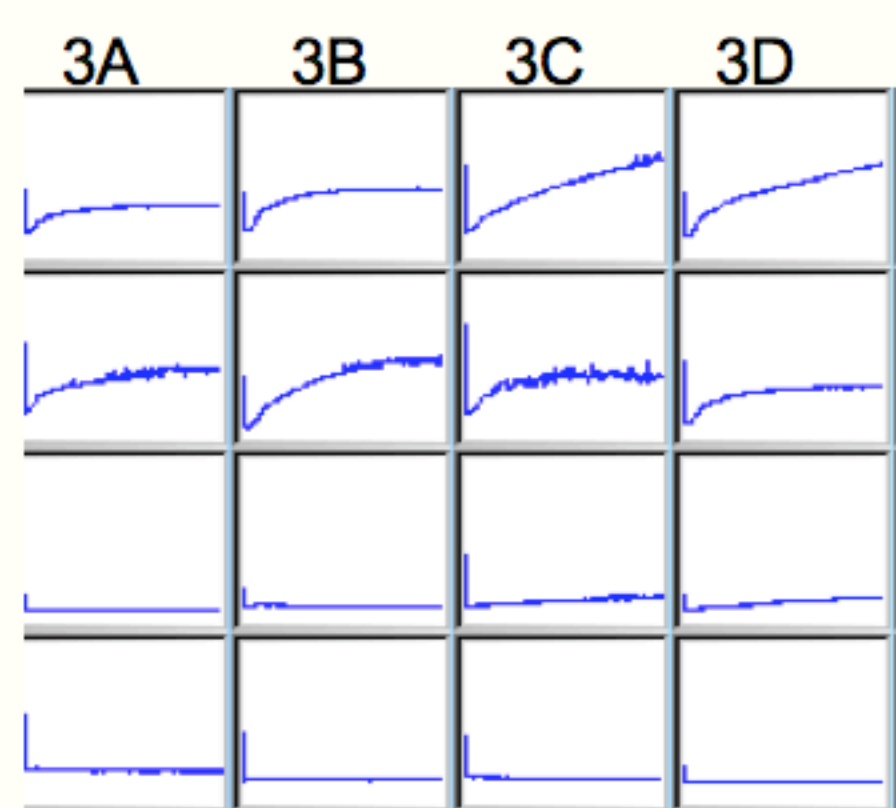


Figure 4: Genetic analysis of the xylose positive phenotype. 14 xylose positive wine strains produced viable spores. About half of the wine strains were homozygous for xylose utilization, while some had both xylose positive and xylose negative progeny. In a cross to a laboratory strain all exhibited a segregation pattern consistent with single gene inheritance. Shown to the left are the data for one tetrad in the same media as described in the legend to Figure 2.

Tetrad analysis of crosses between strains put all of the wine strains in the same complementation group.

Sub-telomeric regions of chromosomes 15R and 16L are nearly identical; Simi white wine strain has undergone recombination in *FDH1* locus

(as determined by inverse PCR sequencing)

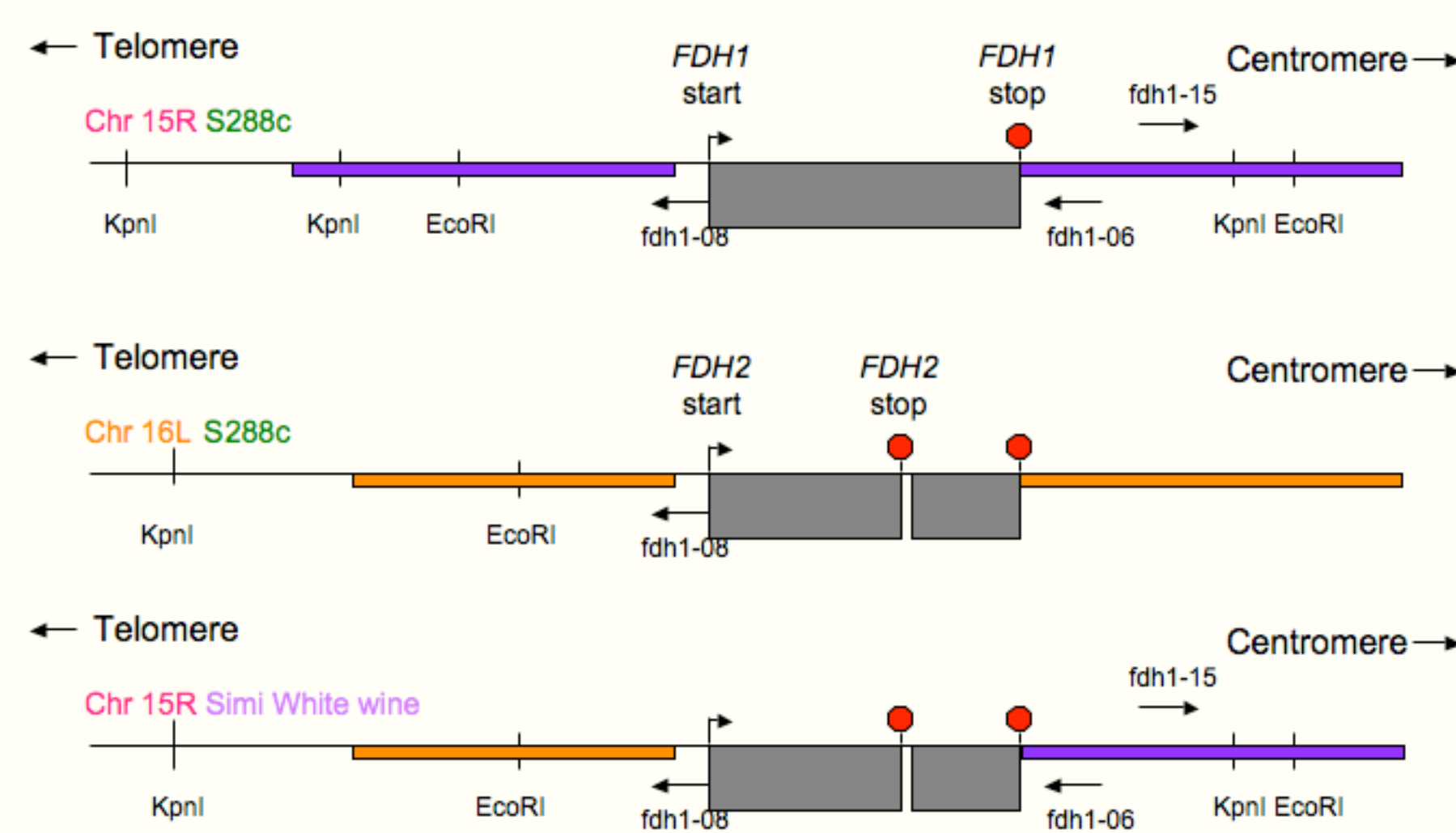


Figure 7: The paralogous recombination event between chromosomes 15 and 16 has been detected in all wine strains tested, including African palm wine strains (but not in sake or ale yeast strains). Since only about 10% of wine strains exhibited ability to utilize xylose, this event is not solely responsible for the phenotype. We have generated sequences for *FDH1* coding and promoter regions for multiple xylose positive and negative wine strains. Presence of the stop codon in *FDH1* is not shared by all xylose positive strains, suggesting that independent recombination events lead to the rearrangement.

Conclusions

- We have identified many naturally occurring yeast strains with some ability to utilize xylose.
- All xylose positive *S. cerevisiae* strains are either wine strains, or associated with vineyards.
- All *S. cerevisiae* positive strains have a single gene in the same complementation group responsible for the phenotype.
- The gene lies close to the right telomere in chromosome 15, and is likely *FDH1*, or *YOR389W*.
- A paralogous region on chromosome 16 undergone recombination with this region in many of the strains for which we have generated sequence.