A role for *MED15* in the domestication of wine yeast

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Project Summary

Question: Is there a role for the global transcriptional regulator, Med15 in the domestication of wine yeast?

Approach: We isolated wine yeast alleles of *MED15* which vary in the length of three polyglutamine tracts. Each allele was sequenced and introduced into the lab strain. The new strains were characterized in three ways: (i) tolerance to fermentation stresses; (ii) fermentation efficiency and (iii) expression of relevant genes. The relationship between phenotype and genotype was evaluated.

Results: Strains lacking *MED15* exhibited reduced fermentation and fermentation stress response while some *MED15* alleles from wine yeast improved fermentation, altered the expression of fermentation genes, and improved acetic acid and ethanol tolerance of the lab strain.

Significance: Variable length microsatellite tracts in Med15 and other global transcriptional regulators may facilitate the adaptation of unicellular organisms to novel environments by generating transcriptome variants, some of which will impart important resiliency traits.

Domestication niches of *S. cerevisiae*: Wine Yeast have adapted to fermentation stresses

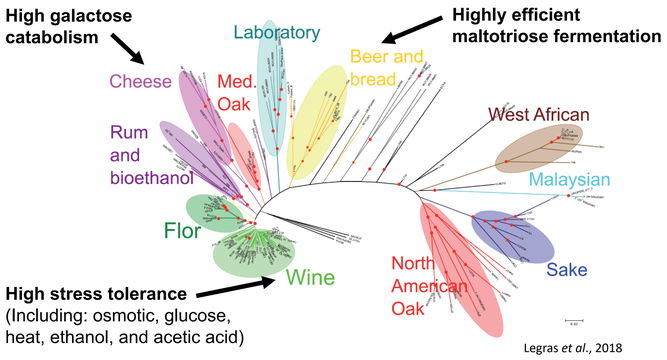

Figure 1: *S. cerevisiae* wine yeast form a distinct clade whose members have been domesticated to ferment grape juice effectively. *S. cerevisiae* (bakers/brewers yeast) is a diverse species found in many different environments. Distinct clades contain closely related strains that are adapted to specific environments. Stresses associated with wine fermentation include: limited nitrogen, osmotic stress (high concentration of glucose in grape must), ethanol toxicity, and acetic acid/low pH stress. Phenotypic data for wine and beer yeast from (Gallone *et al.*, 2016).

Med15 regulates flocculation and fermentation associated stress responses in flor and laboratory yeast

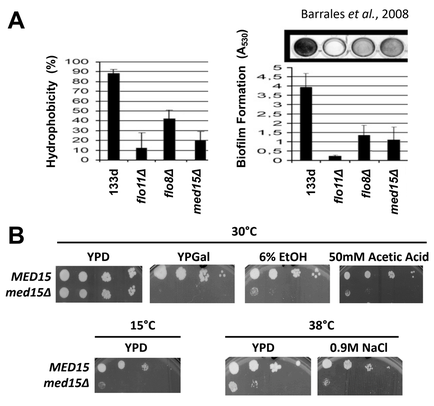

Figure 2: General and fermentation associated stress responses regulated by *MED15*. A) Flocculation of yeast cells is one response to fermentation stresses. Flocculation phenotypes (hydrophobicity and biofilm formation) of flor-laboratory hybrid yeast strain 133d depend on flocculation genes (*FLO11* (flocculin/adhesin) & *FLO8* (transcription factor)) as well as *MED15* (Data from Barrales *et al.*, 2008). Hydrophobicity was measured as the loss of cells from the aqueous layer following an octane extraction. Biofilm formation was measured by crystal violet staining of biofilms in microtiter dish. **B)** Spot assays of *MED15*-dependent stress response phenotypes. 10-fold serial dilutions of wild type *MED15* and *med15Δ* log phase cultures spotted on YPD media or media containing galactose, 6% ethanol, 50mM acetic acid, or 0.9M NaCl at 15, 30, or 38°C.

Yeast Med15 is a glutamine-rich subunit of the tail module of the RNA Pol II Mediator complex

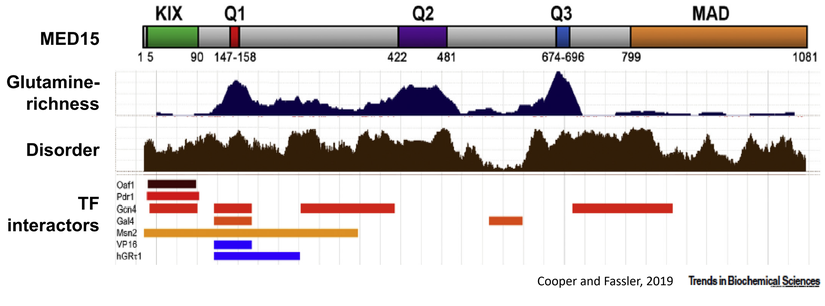

Figure 3: Med15 is a glutamine-rich and intrinsically disordered transcriptional regulator which functions by interacting with DNA bound transcription factors. Cartoon depiction of the major features of the Med15 protein including the amino acid coordinates of the KIX (CREB binding) domain, polyglutamine (poly-Q) tracts Q1, Q2, Q3, and Mediator Association Domain (MAD). Glutamine-rich regions are depicted as the percentage of glutamine residues in sliding windows of 40 amino acids. Protein disorder prediction using IUPRED, which characterizes the tendency of a given amino acid to fall into an ordered or disordered region. Experimentally determined amino acid intervals within Med15 known to be important for interaction with the indicated yeast (Oaf1, Prd1, Gcn4, Gal4, and Msn2), viral (VP16), and human (glucocorticoid receptor, hGRτ1) transcription factors (TFs) (Reviewed in Cooper and Fassler, 2019).

References

Barrales R. R. (2008) **178**(1)
Cooper D. G. and Fassler J. S. (2019) *TIBS* **44**(9)
Gallone B. *et al.* (2016) *Cell* **166**(6)
Legras J. L. *et al.* (2018) *Mol Biol Evol* **35**(7)

Acknowledgements

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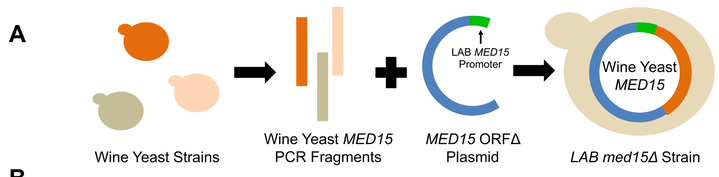
Wine yeast *MED15* alleles with Q-tract polymorphisms were introduced into the lab strain



B

Strain/Allele	Designation	Description	SNPs		Q-tract polymorphisms Q1	Q2	Q3
LAB	S288c	Lab	Syn	Non	12	10(2)17	23
WY7	Lalvin ICV D254	Red and white grape	1	2	21	11(1)17	25
WY15	DSM Fermichamp	Fermentation booster	1	5	28	9(2)18	25
WY20	Kyokai 7	Sake	7	6	21	10(2)16	27
WY23	NRRL Y-17772	Palm wine	6	3	21	12(0)0	27

Figure 4: *MED15* alleles from wine yeast were introduced into the lab strain to study the influence of polyglutamine tract polymorphisms. A) *MED15* alleles from four wine yeast (WY) strains were introduced into the *med15Δ* lab strain on a plasmid containing *MED15* regulatory sequences from the lab strain. **B)** Strain/Allele, names assigned for reference. Designation, common names of the source strains of the *MED15* alleles. Description, the common use of each strain. SNPs, number of synonymous (Syn) and non-synonymous (Non) base pair changes relative to LAB. Q-tract polymorphisms, number of glutamine repeats for Q1 and Q3 and glutamine-alanine dimeric repeats for Q2. Q2 notation 10(2)17 corresponds to the sequence QA[10]QAA[2]QA[17]. Different tract lengths are emphasized with colored text.

Wine and beer yeast Med15 polyglutamine tracts are associated with fermentation domestication phenotypes

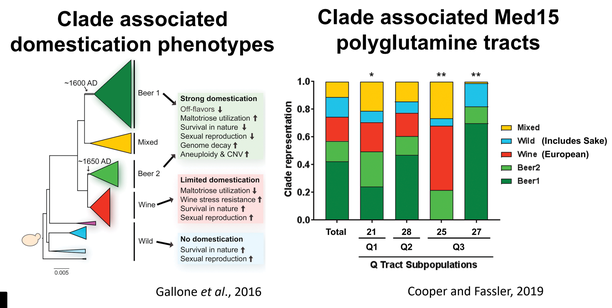


Figure 5: Association between Med15 polyglutamine tract lengths and niche-specific phenotypes. Left) Condensed maximum likelihood phylogeny of 181 *S. cerevisiae* strains (Tree from Gallone *et al.*, 2016). Beer and wine yeast display distinct metabolic, fermentation, and stress response phenotypes. **Right)** Histogram showing the proportion of yeast strains that are found within Med15 poly-Q tract subpopulations. The composition of each tract length population was compared to the expected composition based on random/equal representation using chi-squared goodness of fit test (n=104) *p<0.001, ** p<0.0001.

Hypotheses

1. *MED15* regulates fermentation in a polyglutamine tract length dependent manner.
2. *MED15* alleles from wine yeast are optimized for grape juice fermentation associated activity by changes in the length of polyglutamine tracts. ‘
3. *MED15* alleles from wine yeast will improve the fermentation and grape juice stress response in the lab strain.

WY *MED15* alleles are compatible with the lab strain: Specific alleles improve acetic acid and ethanol tolerance

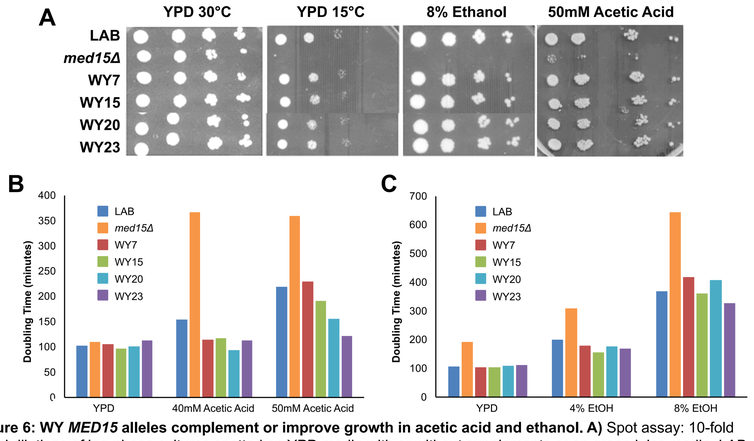


Figure 6: WY *MED15* alleles complement or improve growth in acetic acid and ethanol. A) Spot assay: 10-fold serial dilutions of log-phase cultures spotted on YPD media with or without supplements or on grape juice media. LAB or WY *MED15* alleles in the deletion lab strain. **B & C)** A comparison of doubling time for *med15* deletion strains expressing LAB or WY *MED15* alleles. Growth was measured in YPD media with or without acetic acid (40mM or 50mM) (B) or ethanol (4% or 8%) and plotted as doubling times (C). The *med15Δ* strain has low tolerance of acetic acid and ethanol. The WY20 and WY23 alleles, which share a Q3 tract of 27 glutamines, confer improved acetic acid tolerance. The WY15 allele confers improved ethanol tolerance.

MED15 regulates fermentation

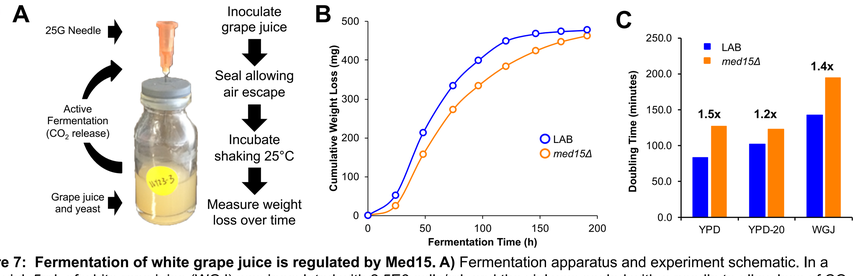


Figure 7: Fermentation of white grape juice is regulated by Med15. A) Fermentation apparatus and experiment schematic. In a small vial, 5mL of white rape juice (WGJ) was inoculated with 2.5E6 cells/mL and the vial was sealed with a needle to allow loss of CO_2 gas. Vials were incubated at 25°C in a shaking incubator and measurements of weight lost were made over time. Lost weight from CO_2 release serves as a measure of fermentation. **B)** Cumulative weight loss over 8 days for LAB and *med15Δ* strains in WGJ. **C)** Growth, depicted with doubling times, of LAB (*MED15*) and *med15Δ* strains in rich media containing 2% glucose (YPD), YPD supplemented with an additional 20% glucose (YPD-20), and white grape juice (WGJ) at 22°Bx supplemented with leucine, adenine, lysine, and uracil. The *med15Δ* strain maintains slightly longer doubling time than WT (1.2x-1.5x) across all media tested.

Wine Yeast *MED15* alleles (15 and 23) improve the fermentation activity of the lab strain

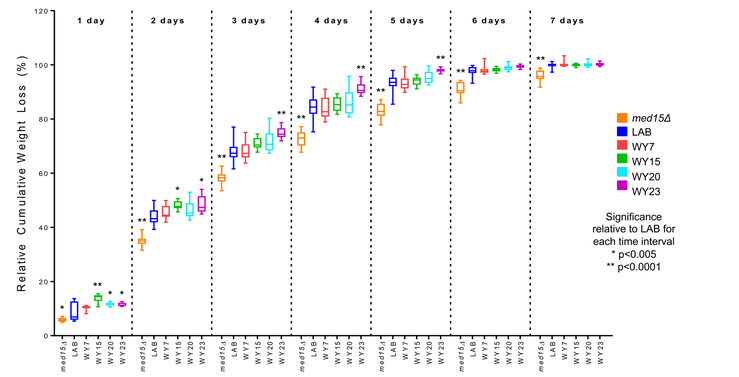


Figure 8: Fermentation of white grape juice is regulated by Med15. Relative cumulative weight loss over 7 days for LAB, *med15Δ*, and WY strains in WGJ. Fermentation experiments conducted in small vials as in Figure 7A. Statistical differences determined by one-way ANOVA for each time interval (n=10).

MED15 regulates ethanol metabolic genes during early stages of fermentation

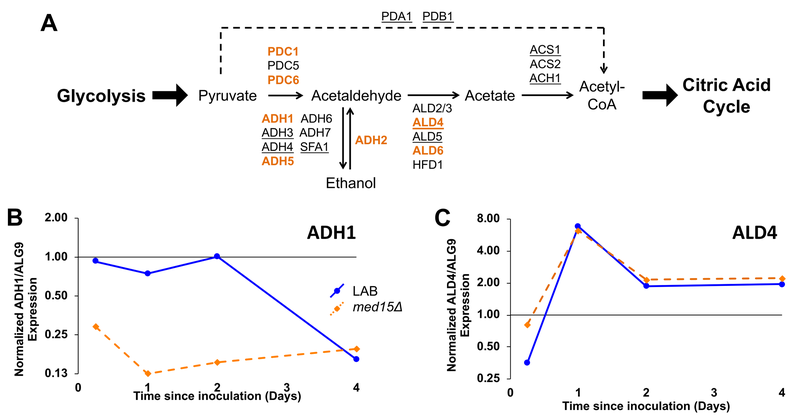


Figure 9: WY Med15 differentially regulates fermentation genes in the lab strain. A) Alcoholic fermentation metabolic pathway and genes. Genes with mitochondrial localized protein products underlined. Genes shown in this study to be differentially regulated by Med15 are highlighted in orange and in bold type. **B-C)** Med15 alters expression of *ADH1* (B) and *ALD4* (C) during fermentation in white grape juice.

Wine Yeast MED15 alleles (7, 15, and 23) increase expression of ethanol metabolic genes

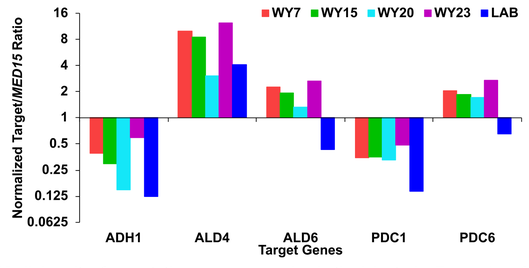


Figure 10: WY Med15 alleles alter fermentation gene expression in the lab strain. Expression of ethanol metabolic genes relative to the LAB allele (royal blue) in log phase YPD cultures. Expression of each gene is normalized to the control transcript, *ALG9* and then to levels of *MED15* in each strain.

Working Model: Polyglutamine tract variants confer altered Med15 fermentation activities

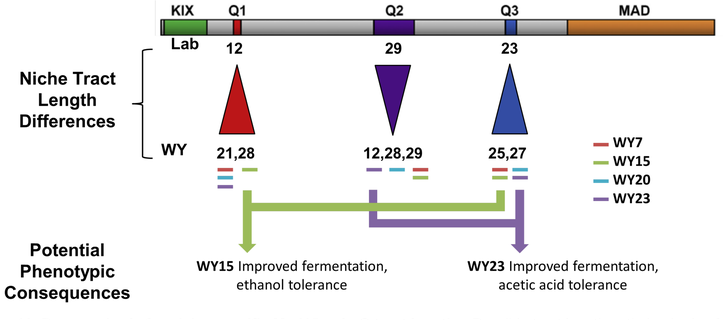


Figure 11: Proposed role for niche-specific Med15 poly-Q tract lengths. Possible tract length optimized roles for specific wine yeast *MED15* allele polyglutamine tracts.

Conclusions:

- Med15 regulates fermentation in addition to fermentation associated stress responses.
- A Q3 tract of 25 corresponds to improved fermentation activity and ethanol tolerance as displayed by the *MED15* allele from WY15.
- A Q3 tract of 27 corresponds to improved fermentation activity and acetic acid tolerance as displayed by the *MED15* allele from WY23.
- The combination of specific Q3 tracts and unique Q1 or Q2 tracts in *MED15* alleles from WY15 and WY23 respectively might be required to produce the observed phenotypes.
- These results suggest a role for polyglutamine tract length variability in the domestication of wine yeast by optimizing the fermentation and stress response activity of the general transcriptional regulator Med15.