Diversity and adaptation of flor yeast : new data for an old question

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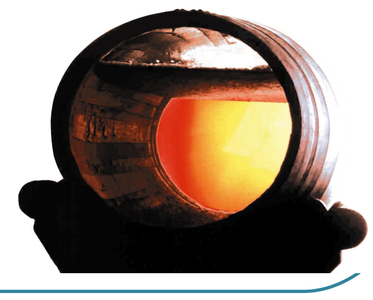
Context and Goals

Saccharomyces cerevisiae, our favorite model organism has been used for millennia for the production of all sort of fermented beverages as well as for bread rising. In contrast with these fermentation lifestyles, during flor wine aging Saccharomyces cerevisiae strains are growing aerobically at the surface of wine as a biofilm. As a significant part of the nutriment has been depleted from wine during alcoholic fermentation, and glucose has been replaced by ethanol, flor yeast must have overcome the different stress imposed by this niche.

This work aims at characterizing Flor strains in order to unravel the basis of flor yeast adaptation to sherry like biological aging.

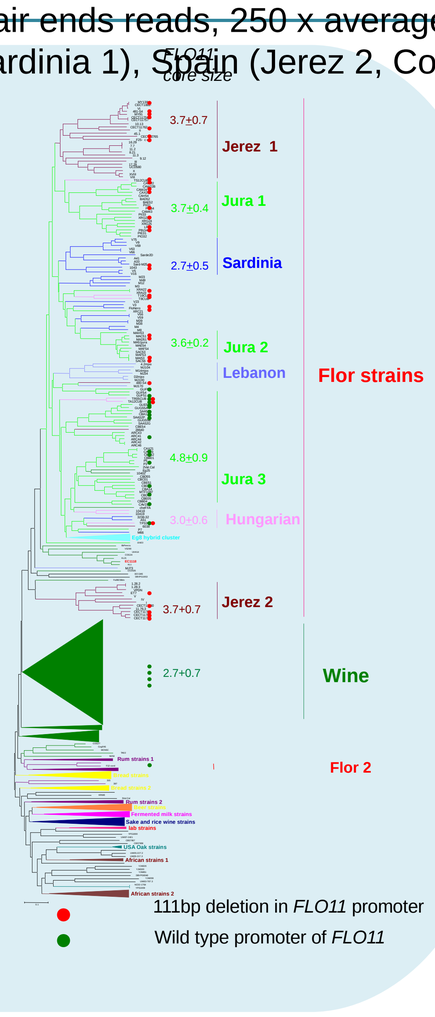


Experimental Approaches

- Diversity of Flor strain from different countries was evaluated with 12 microsatellite markers according to Legras et al 2007 . 119 flor strains (64 from Jura, 30 from Sardinia, 43 from Spain) were genotyped and compared to 500 strains already characterized .
- aCGH on Yeast2 Affymetrix arrays was performed for 6 flor strains from: France (Jura 2), Hungary (Tokay 1), Italy (Sardinia 1), Spain (Jerez 2) in comparison to three wine strains (one classical wine U13, one hybrid Eg8 and one Eg25 flor type wine strains).
- Genome sequencing (Hiseq2000, 100bp pair ends reads, 250 x average coverage) of 8 wine strains and 8 flor strains (France (Jura 2), Hungary (Tokay 2), Italy (Sardinia 1), Spain (Jerez 2, Cordoba 1)). Assemblies were obtained with SOAP and SNP scored with GATK.

Diversity of flor strains evaluated with microsatellites markers

- Flor strains from Hungary, Italy, Spain, and France belong to one main origin and few Spanish strains are clustered in a second group
- Related strains can be encountered in several vineyards (Romania, Lebanon and France (Alsace)).
- FLO11 core size varies according to the clusters. Some clusters do not carry the deletion deletion in the promoting region described by Fidalgo et al. 2006
- Flor strains present a variable ability to sporulate and produce viable spores . Interestingly strains from (Jura3) sporulate nicely whereas Spanish flor strains did not produce viable spores



Genomic specificities of flor strains evaluated with aCGH

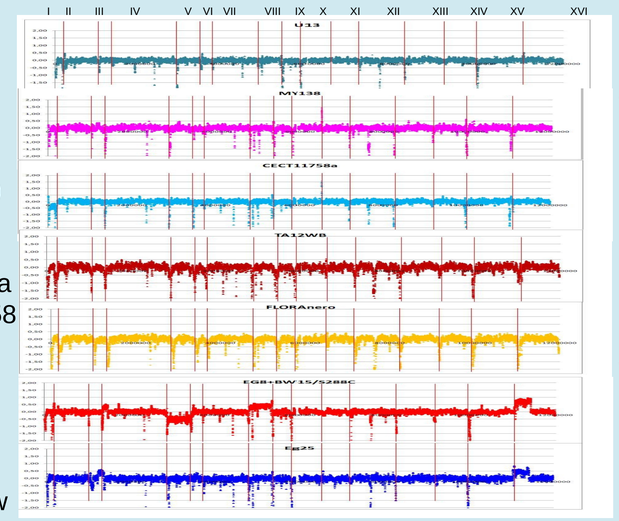
Infante et al 2003 described large aneuploidies when comparing two flor strains that they associated to velum growth adaptation

CNV were detected with DNAcopy Venkatraman et al., 2007 Bioinformatics)

Large aneuploidies can be detected for Eg8 hybrid strain or Eg25 given here as a control, but only for flor strain CECT11758 (Chromosome I)

For each flor strain 31 to 55 genes present a lower hybridization

Only **two genes** YKL222C and YKL221w were amplified in 4 out of 6 flor strains



Flor yeast Genomics preliminary results – HGT introgressions

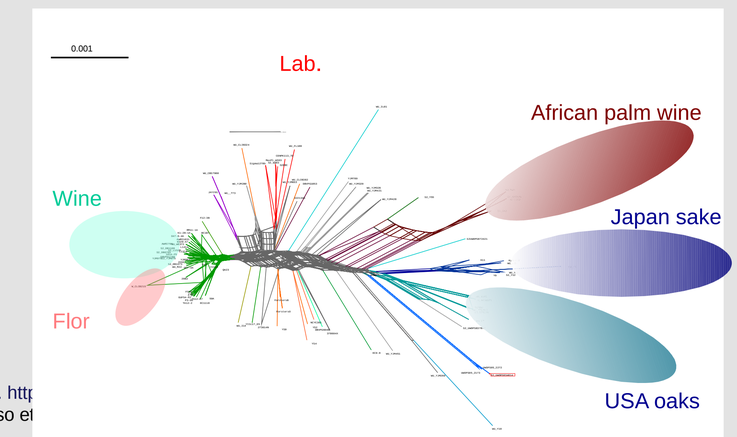
Three HGT caused introgressions (region A BC and) have been characterized in EC1118 that we tried to detect in the genome of Flor strains:

Strain	Region A	Region B	Region C
F25	-	-	-
77	-		
P3	-	-	
Guf54	-		
TA12	-	-	
TS12	-	-	-
Sarde2D	-	-	
F12	-		
EC1118			

- Region A was not detected in sequenced flor strains (but in some other flor genotypes)
- Region B is detected in half of flor strains
- Region C is detected in most flor strain (*FOT1-2* and ***FSY1*** genes)
- Other genes: Ty5-6 of *S. paradoxus* also found in QA23

Global phylogeny of Flor yeast obtained from Genome sequencing

Fig : Phylogenetic Network obtained with SPLITREE from the matrix of number of SNP per kb estimated from the pairwise genome sequence alignment of 95 strains with MUMmer 3.0

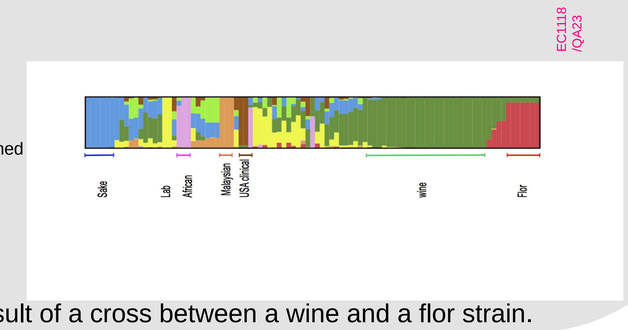


Yeast genomes from Liti et al. 2009, Fay et al. http
Novo et al 2009, Borneman et al 2011, Argueso et

Structure output obtained form a subset of 5000 non ambiguous positions obtained from genomes alignments .

Optimum K =8 evaluated with 10 runs per K according to Evanno's method (2005)

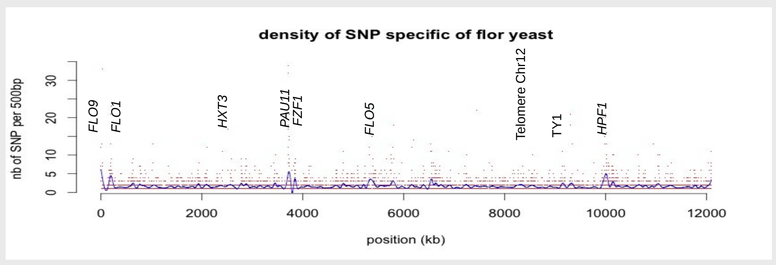
K = 8
(10 runs combined with CLUMP)



EC1118 and QA23 are the result of a cross between a wine and a flor strain.

Density of SNP specific to flor yeast reveals divergent regions

(BWA alignment of reads on S288C genome sequence, SNP call made with genotypes were called with the Genome Analysis Toolkit) 7 flor strains were compared to 8 wine strains



- Detection of different divergent genes (ie *PAU11*, FZF1, *FLO5 -9*, *HXT3* ….)
- Other events: translocations, chimeric genes

Flor *HXT3* allele, has been described from strain Fermichamp: 1 mutation (625 A → G : Ile209 → Val) gives Hxt3p a higher affinity to fructose (Guillaume et *al.,* 2007 *AEM,* Blondin et al unpublished data)

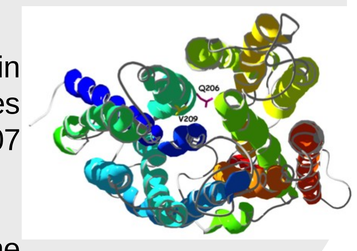


Figure : 3D structure of *HXT3* : V209 is facing Q206 in the hexose channel

Conclusions:

Flor strains are found mainly in one group of *S. cerevisiae* strains, and represent a specific lineage

CNV is very likely not the main mechanism involved in adaptation to velum growth, but polymorphism (translocation, mutations, …) with specific alleles.

Flor strains are more fructophilic than classical wine strains as they carry an improved low affinity transporter for fructose allele of *HXT3* and the high affinity fructose transporter *FSY1* of region C of EC1118 (Galeote et al. 2010).

References:

Legras et al. 2007. Mol Ecol 16, 2091–2102 Fidalgo 2006 PNAS 103, 11228-11233. Infante 2003, Genetics Evanno 2005, MolEcol