Comparative Genomics of Natural *Saccharomyces cerevisiae* x *S. kudriavzevii* Hybrids

David Peris[1], Christian A. Lopes[2,3], Carmela Belloch[2], Amparo Querol[2] and Eladio Barrio[1].

[1]Instituto 'Cavanilles' de Biodiversidad y Biología Evolutiva, Universidad de Valencia, 46071 Valencia, Spain.
[2]Departamento de Biotecnología de Alimentos. Instituto de Agroquímica y Tecnología de los Alimentos. CSIC., 46100 Burjassot, Valencia, Spain.
[3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICYT), Universidad de Comahue, Neuquén, Argentina.

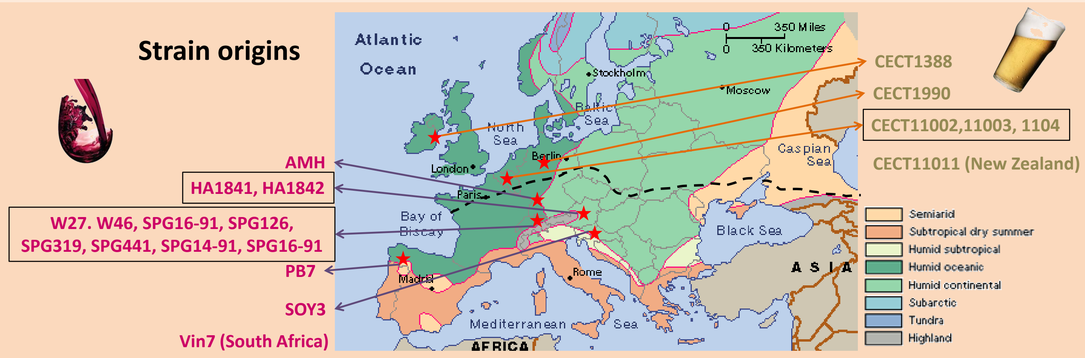
Introduction

The application of molecular characterization methods demonstrated that several beer and wine *Saccharomyces* strains contain genomes composed of different fractions originating from two o more *Saccharomyces* species (interespecific hybrids). Hybrids between *S. cerevisiae* and *S. kudriavzevii* have been found in wine and beer fermentations.

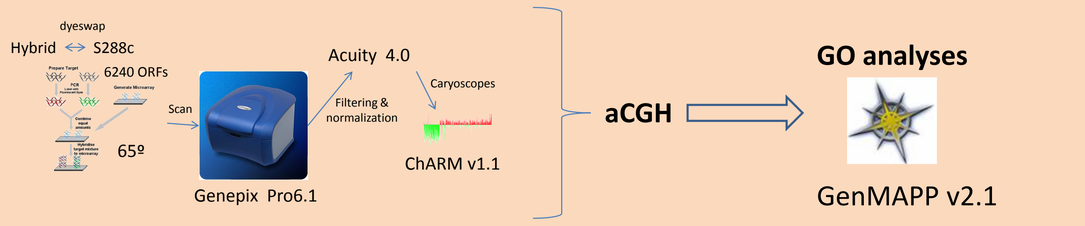
A previous genome characterisation in Swiss wine hybrids, by a combination of RFLP analysis of 35 gene regions, aCGH analysis, ploidy estimation and gene dose determination, indicated that, after hybridization, the hybrid genome underwent extensive chromosomal rearrangements, including chromosome losses and generation of chimerical chromosomes. As a result, hybrid genomes maintained the *S. cerevisiae* genome, but reduced the *S. kudriavzevii* fraction. The role of the *S. kudriavzevii* genome in the hybrids is unclear. Comparative physiological fermentative behavior between *Saccharomyces* species and their hybrids showed that *S. kudriavzevii* is more cold-tolerant than *S. cerevisiae*, whereas good fermentative characteristics such as glucose and ethanol tolerances are superior in the *S. cerevisiae* strains.

In this study new natural *S. cerevisiae* x *S. kudriavzevii* hybrids isolated from different wine and beer fermentations in Europe, South Africa, Australia and Germany are analysed by RFLPs and aCGH. The determination of their genomic structure allowed us to determine their origins and evolution. Also the analysis of the loss and preservation of gene sets coming from the parental *S. cerevisiae* and *S. kudriavzevii* genomes, will help us to elucidate the their contributions to the peculiar advantageous physiological properties exhibited by hybrid strains

Material & Methods

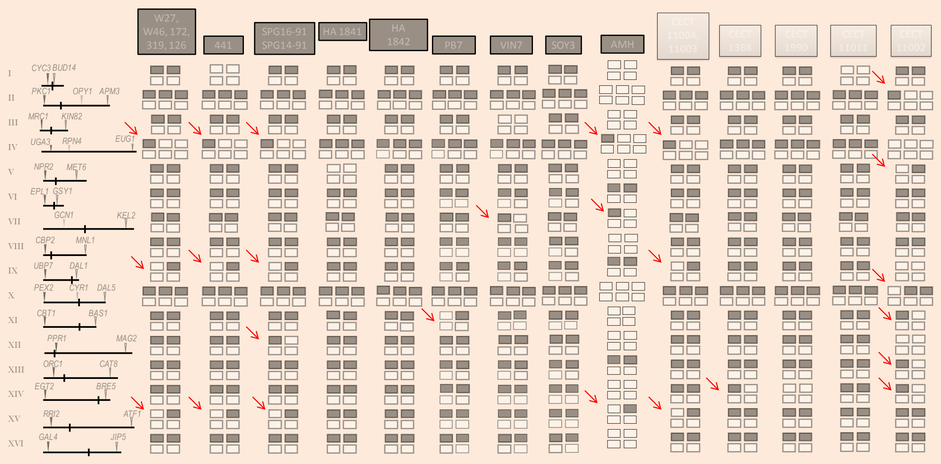


RFLP analysis: DNA extraction according to Querol *et al.* 1992. The procedure for PCR amplification and subsequent restriction analysis of 35 genes in hybrids is described in Gonzalez *et al* (2008).

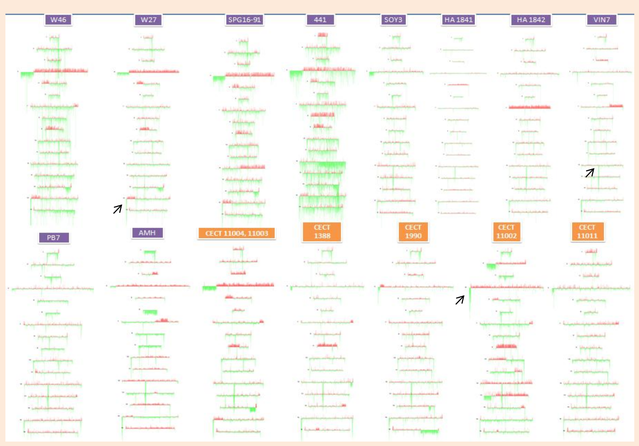


Genome rearrangements clustering analysis: Similarity indices of the shared chromosomal rearrangement events were estimated and used to obtain a UPGMA dendrogram.

Results



RFLP analysis of 35 gene regions in *S. cerevisiae* x *S. kudriavzevii* hybrids. Each square corresponds to a copy of each gene region according to its chromosome location, indicate in the left map. Alleles of *S. cerevisiae* and *S. kudriavzevii* origin are represented by white and black squares, respectively. Red arrows indicate chimerical chromosomes.



Karyoscope representation of the results of the microarray CGH analysis of *S. cerevisiae* x *S.kudriavzevii* hybrids, wine hybrids are labelled in purple and brewing hybrids in orange. The aCGH data is depicted for each chromosome, from chromosome I at the top to XVI at the bottom. Regions with higher red signals correspond to *S. cerevisiae* genes present in more copies than average in the hybrid genome. Regions with higher green signals represent *S. cerevisiae* genes with lower copies than average or absent in the hybrid genome. Black arrows are examples of *S. cerevisiae* gene losses.

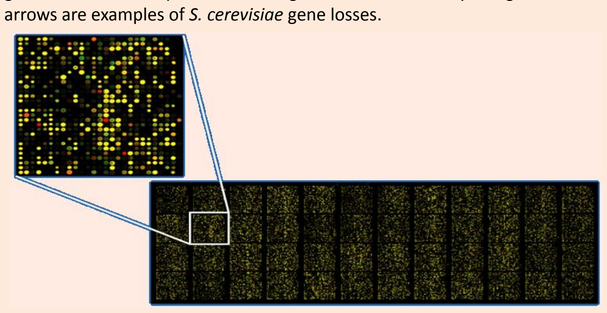


Table 1. Rearrangement events: Summary of the different genome rearrangements present in hybrids.

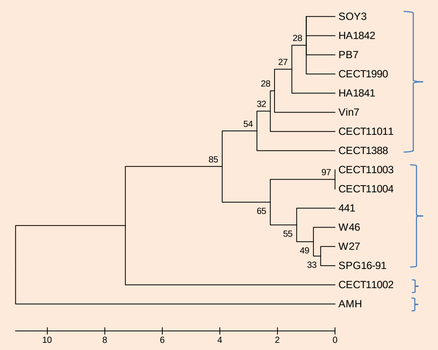
Chromosome	Rearrangments	Strains
I	*S. kudriavzevii's chromosome lost*	441, AMH, CECT 11011
	Doble dosage of *S. cerevisiae* 's chromosome	441, CECT 11011
II	Chimerical chromosome	CECT 11002
	S. kudriavzevii's chromosome lost	AMH
	Doble dosage of *S. cerevisiae* 's chromosome	AMH
III	Chimerical chromosome	AMH
	S. kudriavzevii's chromosome lost	Vin7
	Doble dosage of *S. cerevisiae* 's chromosome	Vin7, CECT11003, CECT 11004
IV	Chimerical chromosome 1	W27, W46, 441, SPG16-91, CECT11003, CECT 11004
	Chimerical chromosome 2	AMH, SOY3
V	Chimerical chromosome 1	W27, W46, 441, SPG16-91, CECT11003, CECT 11004
	Chimerical chromosome 2	CECT 11002
	S. kudriavzevii's chromosome lost	AMH, HA1841
	Doble dosage of *S. cerevisiae* 's chromosome	AMH, HA1841
VII	Chimerical chromosome 1	W46, CECT 11003, CECT 11004,CECT 11002, CECT 11011, CECT1388
	Chimerical chromosome 2	AMH, Vin7
	Doble dosage of *S. cerevisiae* 's chromosome	HA1837
VIII	*S. kudriavzevii's chromosome lost*	AMH
IX	Chimerical chromosome	W27, W46, 441, SPG16-91, CECT11003, CECT 11004
	S. kudriavzevii's chromosome lost	CECT 11002
	Doble dosage of *S. cerevisiae* 's chromosome	CECT 1388, CECT 11002
X	Chimerical chromosome	CECT 11002
	S. kudriavzevii's chromosome lost	AMH
	Doble dosage of *S. cerevisiae* 's chromosome	AMH
XI	Chimerical chromosome 1	CECT 11002
	Chimerical chromosome 2	PB7
	S. kudriavzevii's chromosome lost	AMH
	Doble dosage of *S. cerevisiae* 's chromosome	AMH
XII	Chimerical chromosome 1	SPG16-91
	Chimerical chromosome 2	CECT 1388
	S. kudriavzevii's chromosome lost	AMH, CECT 11002
	Doble dosage of *S. cerevisiae* 's chromosome	AMH, CECT 11002
XIII	Chimerical chromosome	CECT 11002
XIV	Chimerical chromosome 1	W27, W46, 441, SPG16-91, CECT11003, CECT 11004
	Chimerical chromosome 2	CECT 1388, CECT 11002
	S. kudriavzevii's chromosome lost	AMH, CECT 1990
	Doble dosage of *S. cerevisiae* 's chromosome	AMH
XV	Chimerical chromosome	W27, W46, 441, SPG16-91, CECT11003, CECT 11004
XVI	Chimerical chromosome	CECT 1990
	S. kudriavzevii's chromosome lost	AMH
	Doble dosage of *S. cerevisiae* 's chromosome	AMH

Table 2. Gene Ontology analysis. significant GO terms are indicated for those most interesting *S. kudriavzevii* genes maintained in wine or brewing hybrids or common to all hybrids.

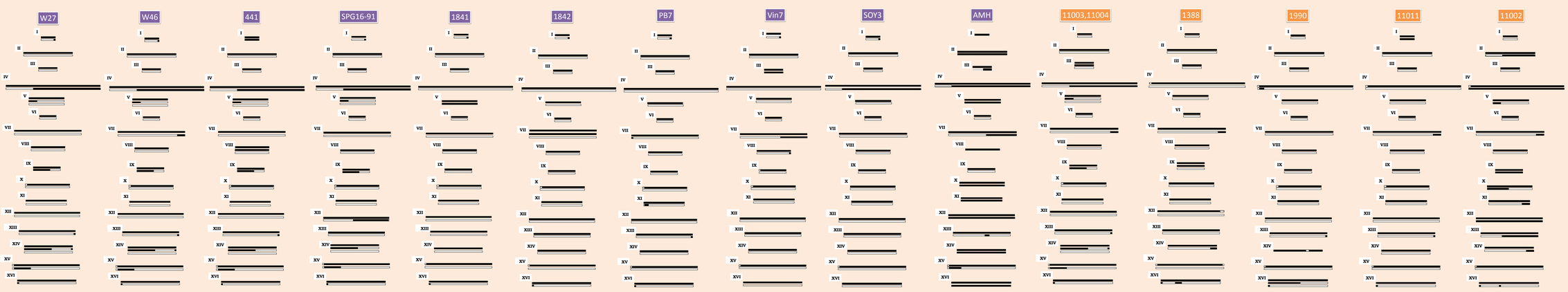
	GOID	GO Name	$N_{changed}/N_{measured}$ (%)	PermuteP
WINE				
Biological process	6487	protein amino acid N-linked glycosylation	36/42 (85.7)	0.013
Metabolic pathways		Ergosterol_Biosynthesis	17/19 (89.5)	0.049
BREWERING				
Biological process	6487	protein amino acid N-linked glycosylation	28/42 (66.7)	0.017
Metabolic pathways		tRNA_Synthetases	23/35 (65.7)	0.026
		Glycine_serine_and_threonine_metabolism	27/42 (64.3)	0.03
		Mitochondrial_tRNA_Synthetases	11/14 (78.6)	0.03
		Fatty_Acid_Elongation_Saturated	4/4 (100)	0.039
		Arginine_and_proline_metabolism	16/23 (69.6)	0.042
		Sulfur_metabolism	9/11 (81.8)	0.046
		Sulfur_Degradation	4/4 (100)	0.048
COMMON				
Molecular function	16668	oxidoreductase activity\, acting on sulfur group of donors\, NAD or NADP as acceptor	5/6 (83.3)	0.038
Biological process	9435	NAD biosynthesis	7/10 (70)	0.047
	6487	protein amino acid N-linked glycosylation	25/42 (59.5)	0.003
Metabolic pathways		Glutamate_metabolism	15/27 (55.6)	0.046
		Sulfur_metabolism	8/11 (72.7)	0.021
		Mitochondrial_tRNA_Synthetases	9/14 (64.3)	0.048
		NAD_Salvage_Pathway	5/6 (83.3)	0.027
		Sulfate_Assimilation_Pathway_II	5/6 (83.3)	0.019
		tRNA_Synthetases	19/35 (54.3)	0.027

Table 3. *S. cerevisiae* gene losses. *S. cerevisiae* gene losses common to all hybrids. Ty elements and non-annotated genes were not included.

Systematic ID	Function_gene
AAD15	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase
AAD16	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase
AAD6	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase, involved in the oxidative stress response
ASP3-1	Cell-wall L-asparaginase II, involved in asparagine catabolism
ASP3-2	Cell-wall L-asparaginase II, involved in asparagine catabolism
ASP3-3	Cell-wall L-asparaginase II, involved in asparagine catabolism
ASP3-4	Cell-wall L-asparaginase II, involved in asparagine catabolism
BDS1	Bacterially-derived sulfatase required for use of alkyl- and aryl-sulfates as sulfur sources
BRR2	RNA-dependent ATPase RNA helicase (DEIH box)
BST1	GPI inositol deacylase of the ER that negatively regulates COPII vesicle formation, prevents production of vesicles with defective subunits, required for proper discrimination between resident ER proteins and Golgi-bound cargo molecules
CUP1-1	Metallothionein, binds copper and mediates resistance to high concentrations of copper and cadmium
CUP1-2	Metallothionein, binds copper and mediates resistance to high concentrations of copper and cadmium
ENA2	P-type ATPase sodium pump, involved in Na+ efflux to allow salt tolerance
ENA5	Protein with similarity to P-type ATPase sodium pumps, member of the Na+ efflux ATPase family
FET5	Multicopper oxidase, integral membrane protein with similarity to Fet3p
FIT1	Mannoprotein that is incorporated into the cell wall via a glycosylphosphatidylinositol (GPI) anchor, involved in the retention of siderophore-iron in the cell wall
FLO1	Lectin-like protein involved in flocculation, cell wall protein that binds to mannose chains on the surface of other cells, confers floc-forming ability that is chymotrypsin sensitive and heat resistant
FLO5	Lectin-like cell wall protein (flocculin) involved in flocculation, binds to mannose chains on the surface of other cells, confers floc-forming ability that is chymotrypsin resistant but heat labile
FLO9	Lectin-like protein with similarity to Flo1p, thought to be expressed and involved in flocculation
MST27	Putative integral membrane protein, involved in vesicle formation
MST28	Putative integral membrane protein, involved in vesicle formation
PRM8	Pheromone-regulated protein with 2 predicted transmembrane segments and an FF sequence, a motif involved in COPII binding
PRM9	Pheromone-regulated protein with 3 predicted transmembrane segments and an FF sequence, a motif involved in COPII binding
RAS1	GTPase involved in G-protein signaling in the adenylate cyclase activating pathway, plays a role in cell proliferation
TDA8	Putative protein of unknown function; YAL064C-A is not an essential gene



UPGMA dendrogram based in the fraction of shared rearrangements between hybrid pairs according to Table 1. Four groups of hybrids were observed.



Genome compositions of hybrids. The *S. cerevisiae* fractions of the hybrid genomes are depicted in black, and the *S. kudriavzevii* fractions in white. Grey bars correspond to *S. bayanus* regions present in the AMH hybrid genome. Wine and brewing hybrids are labelled in purple and orange, respectively.

Conclusions

- By using different techniques we were able to determine the genome composition of natural hybrid yeasts. AMH resulted to be a triple hybrid containing genome fractions from *S. bayanus, S. cerevisiae* and *S. kudriavzevii*.
- At least four different events of recombination gave place to the different hybrids under analysis. Two brewing hybrids isolated from Belgian Trappist beers (CECT11003 and CECT11004) are very similar to Swiss wine hybrids.
- Chimerical chromosomes were generated by recombination between homeologous chromosomes at conserved regions such as ARS sequences, Ty elements, Y' elements, rRNA coding regions, and conserved coding genes.
- Some of these rearrangements are common to hybrids originated by different hybridization events, suggesting the presence of recombination hot spots.
- Hybrid strains share the absence of *S. cerevisiae* genes wine strains like RM11-1a, EC1118 and other *S. cerevisiae* studied by Carreto *et al.* 2008. These results indicate that *S. cerevisiae* parental strain of hybrids was a wine *S. cerevisiae.*
- S*. cerevisiae* x *S. kudriavzevii* hybrids maintained most of the *S. cerevisiae* genome fraction, the only genes absent with respect to the reference laboratory strain S288c are also absent in several wine and vinyard *S. cerevisiae* strains, such as RM11-1a, EC1118, AWRI1631. However, some hybrids lost several chromosomes or large chormosome regions from *S. kudriavzevii*. The most extreme case is strain AMH, which maintained only 3 complete *S. kudriavzevii* chromosomes and 3 regions in chimerical chromosomes.
-Hybrids maintained *S. kudriavzevii* genes involved in stress response (pH, osmotic, oxidative, ethanol and low temperature stresses).

Hybridization between yeast species is a common event and its consequences are the generation of strains better adapted to fluctuating environmental conditions, as those present in biotechnological processes