Two genomes one organism:
Saccharomyces cerevisiae x *S. kudriavzevii* Hybrids

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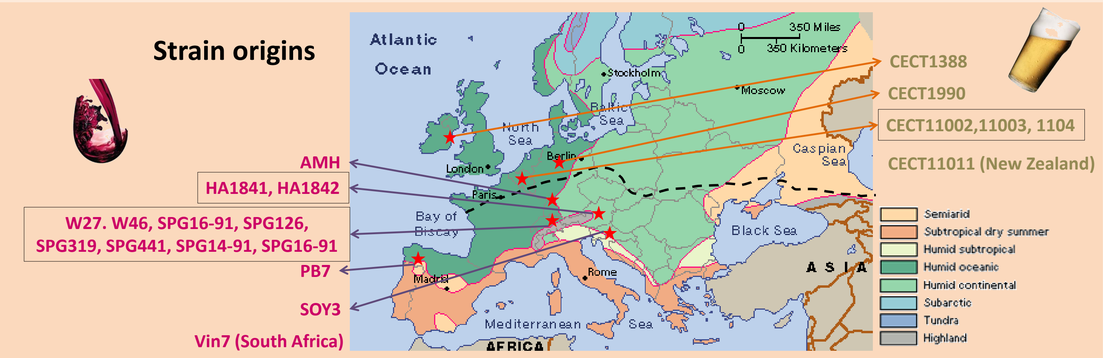
Introduction

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.

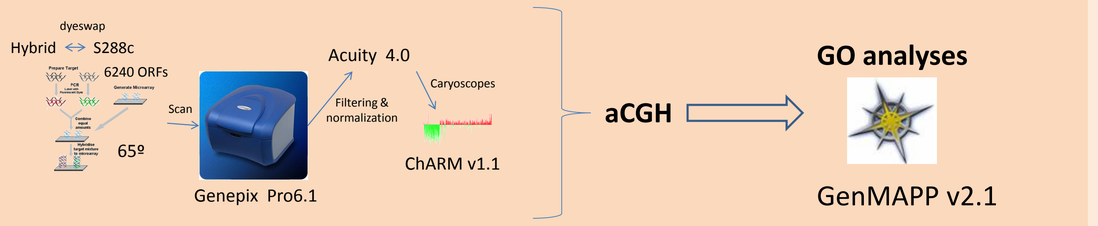
A previous genome characterisation in Swiss wine hybrids, by a combination of RFLP analysis of 35 gene regions, aCGH analysis, ploidy estimation and gene dose determination, indicated that, after hybridization, the hybrid genome underwent extensive chromosomal rearrangements, including chromosome losses 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

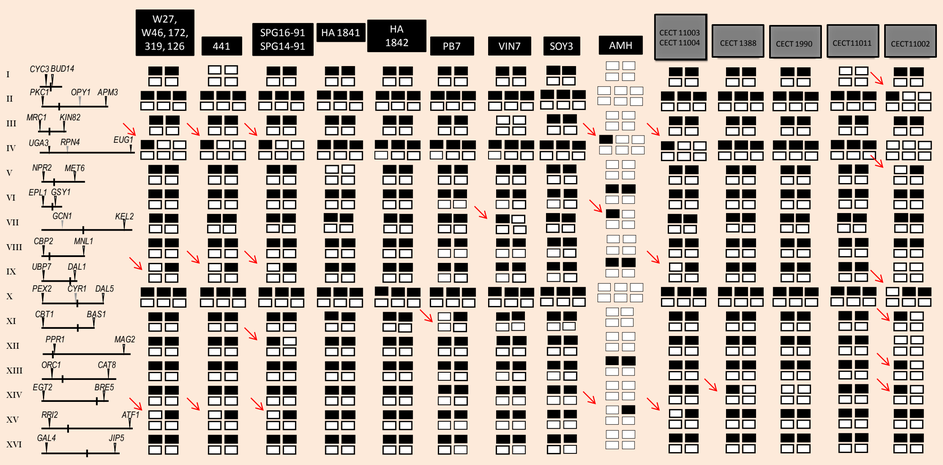


RFLP analysis: DNA extraction according to Querol *et al.* 1992. The procedure for PCR amplification and subsequent restriction analysis of 35 genes in hybrids is described in Gonzalez *et al* (2008).



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.

Table 1. Rearrangement events: Summary of the different genome rearrangements present in hybrids. DD: Doble dosage, CC:Chimerical chromosome (number refers to the type), CHR: Cluster Homology Regions.

Chr	Rearrangements	Strains	Breakpoint	Possible recombining sequences
I	*S. kudriavzevii*'s chromosome lost	441, AMH, CECT 11011		
	DD of *S. cerevisiae*'s chromosome	441, CECT 11011		
II	CC	CECT 11002	YBL018C-YBL011W	Ty1 LTRs, Ty3 LTRs, tRNA-Ile, tRNA-Gly, ARS
	S. kudriavzevii's chromosome lost	AMH		
	DD of *S. cerevisiae*'s chromosome	AMH		
III	CC	AMH	YCR047C-YCR039C	ARS
	S. kudriavzevii's chromosome lost	Vin7		
	DD of *S. cerevisiae*'s chromosome	Vin7, CECT11003, CECT 11004		
IV	CC 1	W27, W46, 441, SPG16-91, CECT11003, CECT 11004	YDL095W	PMT1 (Belloch C et al 2009)
	CC 2	AMH, SOY3	YDL185W-YDL179W	CHR 12
	CC 3	CECT 1990	YDL185W-YDL179W	CHR 12
V	CC 1	W27, W46, 441, SPG16-91, CECT11003, CECT 11004	YER006W	NUG1 (Belloch C et al 2009)
	CC 2	CECT 11002	YEL018C-YEL011W	Ty1 LTRs, Ty4 LTR, tRNA-Gln
	S. kudriavzevii's chromosome lost	AMH, HA1841		
	DD of *S. cerevisiae*'s chromosome	AMH, HA1841		
VII	CC 1	W46, CECT 11003, CECT 11004,CECT 11002, CECT 11011, CECT1388	YGR249W-YGR244C	ARS, CHR 29
	CC 2	AMH	YGR062C-YGR058W	CHR 30
	CC 3	Vin7	YGR106C-YGR112C	tRNA-Leu, tRNA-Lys, Ty1 LTRs, tRNA-Cys, Ty3 LTRs, ARS
	DD of *S. cerevisiae*'s chromosome	HA1842		
VIII	*S. kudriavzevii*'s chromosome lost	AMH		
IX	CC	W27, W46, 441, SPG16-91, CECT11003, CECT 11004	YIL053W	RHR2-RPL34B (Belloch C et al 2009)
	S. kudriavzevii's chromosome lost	CECT 11002		
	DD of *S. cerevisiae*'s chromosome	CECT 1388, CECT 11002		
X	CC	CECT 11002	YJL039C-YJL036C	tRNA-Asp, tRNA-Arg, Ty1 LTRs, ARS, tRNA-Val
	S. kudriavzevii's chromosome lost	AMH		
	DD of *S. cerevisiae*'s chromosome	AMH		
XI	CC 1	CECT 11002	YKR025C-YKR028W	Ty1 LTRs
	CC 2	PB7	YKL203C-YKL204W	ARS
	S. kudriavzevii's chromosome lost	AMH		
	DD of *S. cerevisiae*'s chromosome	AMH		
XII	CC 1	SPG16-91	YLR156W	Cluster of RDN genes (Belloch C et al 2009)
	CC 2	CECT 1388	YLR437-YLR439W	ARS
	S. kudriavzevii's chromosome lost	AMH, CECT 11002		
	DD of *S. cerevisiae*'s chromosome	AMH, CECT 11002		
XIII	CC	CECT 11002	YML012C-YML009W-B	CEN13, ARS
XIV	CC 1	W27, W46, 441, SPG16-91, CECT11003, CECT 11004	YNR001C	CEN14 (Belloch C et al 2009)
	CC 2	CECT 1388, CECT 11002	YNR029C-YNR032W	ARS
	S. kudriavzevii's chromosome lost	AMH, CECT 1990		
	DD of *S. cerevisiae*'s chromosome	AMH		
XV	CC	W27, W46, 441, SPG16-91, CECT11003, CECT 11004, AMH	YOL053W	THI20-PSH1 (Belloch C et al 2009)
XVI	CC	CECT 1990	YPR007C-YPR011C	Ty1 LTRs, tRNA-Gly, tRNA-Lys,
	S. kudriavzevii's chromosome lost	AMH		
	DD of *S. cerevisiae*'s chromosome	AMH		

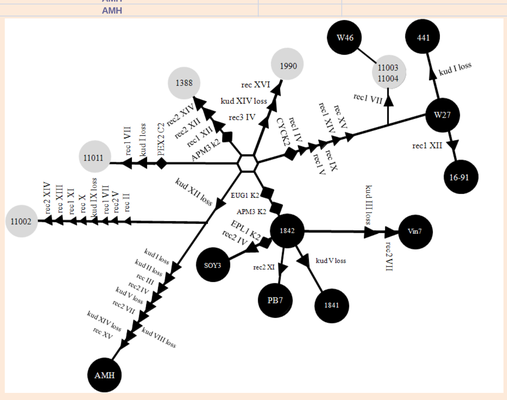
Karyoscope representation of the results of the microarray CGH analysis of *S. cerevisiae* x *S.kudriavzevii* hybrids, wine hybrids are labelled in purple and brewing hybrids in orange. The aCGH data is depicted for each chromosome, from chromosome I at the top to XVI at the bottom. Regions with higher 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 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.

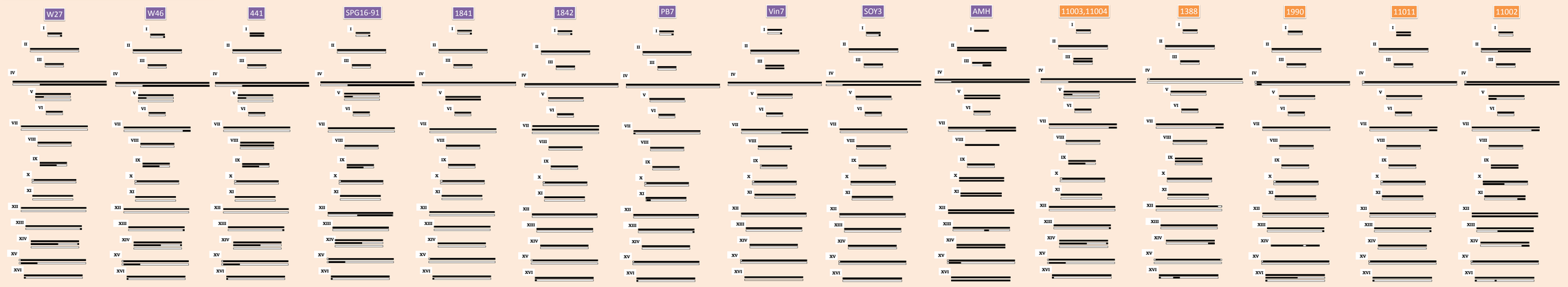
Systematic ID	Function_gene
AAD15	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase
AAD16	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase
AAD6	Putative aryl-alcohol dehydrogenase with similarity to P. 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



Parsimony Tree based in the fraction of shared rearrangements and RFLPs data between hybrid pairs according to Table 1.

Hybrids with *S. kudriavzevii* mitochondrial genome maintain 45% of mitochondrial proteins codified by *S. kudriavzevii* genes.

AMH maintains 29%.



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. Almost of hybrids maintain around 80% of genes coming from *S. kudriavzevii* parental (except CECT11002, 57% and AMH only 29%). All hybrids except AMH maintain a *S. kudriavzevii* mitochondrial genome.

Conclusions

- By using different techniques we were able to determine the genome composition of natural hybrid yeasts.
- At least four different events of hybridization 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*. Lopes C. *et al* 2010 have shown that the RFLPs pattern of *S. kudriavzevii* isolated from Spain are close to the RFLPs alleles in hybrids coming from *S. kudriavzevii* parental that denotes that *S. kudriavzevii* from EU is the parental of these hybrids.
- 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. It seems to be an evolutionary constriction to maintain genes of *S. kudriavzevii* when the mitochondrial genome is coming from *S. kudraivzevii* parental.
-Hybrids maintained *S. kudriavzevii* genes involved in stress response (pH, osmotic, oxidative, ethanol and low temperature stresses) and new studies show that almost of these proteins can formed PPIs among themselves. The question is if these proteins can forme PPIs among *S. cerevisiae* proteins in the hybrid organism.

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