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► To cite this version:

Maite Novo, Frederic Bigey, Emmanuelle Beyne, Virginie Galeote, Frédéric Gavory, et al.. Eukaryote-to-eukaryote gene transfer events revealed by the genome sequence of the wine yeast *Saccharomyces cerevisiae* EC1118. *Proceedings of the National Academy of Sciences of the United States of America*, 2009, 106 (38), pp.16333-16338. 10.1073/pnas.0904673106 . hal-02668729

HAL Id: hal-02668729

<https://hal.inrae.fr/hal-02668729>

Submitted on 31 May 2020

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Eukaryote-to-eukaryote gene transfer events revealed by the genome sequence of the wine yeast *Saccharomyces cerevisiae* EC1118

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Edited by Jasper Rine, University of California, Berkeley, CA, and approved August 3, 2009 (received for review May 5, 2009)

Saccharomyces cerevisiae has been used for millennia in winemaking, but little is known about the selective forces acting on the wine yeast genome. We sequenced the complete genome of the diploid commercial wine yeast EC1118, resulting in an assembly of 31 scaffolds covering 97% of the S288c reference genome. The wine yeast differed strikingly from the other *S. cerevisiae* isolates in possessing 3 unique large regions, 2 of which were subtelomeric, the other being inserted within an EC1118 chromosome. These regions encompass 34 genes involved in key wine fermentation functions. Phylogeny and synteny analyses showed that 1 of these regions originated from a species closely related to the *Saccharomyces* genus, whereas the 2 other regions were of non-*Saccharomyces* origin. We identified *Zygosaccharomyces bailii*, a major contaminant of wine fermentations, as the donor species for 1 of these 2 regions. Although natural hybridization between *Saccharomyces* strains has been described, this report provides evidence that gene transfer may occur between *Saccharomyces* and non-*Saccharomyces* species. We show that the regions identified are frequent and differentially distributed among *S. cerevisiae* clades, being found almost exclusively in wine strains, suggesting acquisition through recent transfer events. Overall, these data show that the wine yeast genome is subject to constant remodeling through the contribution of exogenous genes. Our results suggest that these processes are favored by ecologic proximity and are involved in the molecular adaptation of wine yeasts to conditions of high sugar, low nitrogen, and high ethanol concentrations.

adaptive evolution | comparative genomics | horizontal gene transfer | introgression | *Zygosaccharomyces bailii*

The yeast *Saccharomyces cerevisiae* has been associated with human activity for thousands of years. The earliest evidence of winemaking has been dated to $\approx 7,000$ years ago (1). The fermentation of grape juice exposes yeast cells to harsh environmental conditions (high sugar concentration, increasing alcohol concentration, acidity, presence of sulfites, anaerobiosis, and progressive depletion of essential nutrients, such as nitrogen, vitamins, and lipids). These conditions, as well as unwitting selection by man for optimal winemaking traits (fermentation performance, alcohol tolerance, and good flavor production) have generated hundred of strains that are currently used in the wine industry. As a result, wine yeast isolates belong to a well-defined lineage (2–6).

Deciphering the mechanisms that participate to these evolutionary processes and identifying the variations contributing to the properties of wine yeast remain challenging issues. Wine yeasts are often diploid, heterozygous, and homothallic (4, 7, 8). They have a large capacity for genome reorganization through chromosome rearrangements (9–11), promoting rapid adaptation to environmental changes. Comparative genomics is a suitable approach for cataloguing multiple types of sequence

variation between yeast strains. Comparative genome hybridization analysis of several *S. cerevisiae* genomes has resulted in the identification of gene deletions and amplifications common to most wine yeast strains (7, 12). Analyses of the genome sequences of yeast strains of various origins have shown that nucleotide polymorphism may be the main source of phenotypic variation (5, 6, 13–15).

With a view to deciphering the genetic basis of winemaking traits, we determined the complete genome sequence of the diploid commercial wine yeast strain EC1118. The full gene repertoire of EC1118 was established and provided evidence for several gene transfer events, which were analyzed in detail. These findings provide unprecedented insight into the molecular mechanisms contributing to the adaptation of yeast to winemaking.

Results

Genome Sequence and Analysis. The diploid EC1118 genome was sequenced and assembled using a Sanger/pyrosequencing hybrid approach [supporting information (SI) Table S1 and SI Materials and Methods]. Early in the assembly process it became clear that distinct haplotypes could not be obtained in most cases, because heterozygosity levels were very low (approximately 0.2%). An 11.7-Mb high-quality “pseudohaploid” assembly with 31 scaffolds was obtained (Table S1), corresponding to 96.7% of the S288c nuclear genome, as determined from genome alignments. Nucleotide alignments with other *S. cerevisiae* strains (Table S2) revealed a similar level of nucleotide polymorphism between EC1118 and S288c or the clinical isolate derivative YJM789 (46,825 and 47,253, respectively) and, as expected, a much lower level of nucleotide variation compared with the wine yeast derivatives RM11–1a and AWRI1631 (19,142 and 18,315, respectively).

A total of 5,728 ORFs (except dubious and Ty-associated genes) has been predicted for the nuclear genome of EC1118 (Table S3), of which 5,685 are common to EC1118 and S288c. Several of these ORFs are predicted to be affected by frameshifts

Author contributions: F.B., P.W., S.C., and S.D. designed research; M.N., F.B., E.B., V.G., F.G., S.M., B.C., J.-L.L., and S.C. performed research; M.N., F.B., E.B., V.G., J.-L.L., S.C., and S.D. analyzed data; and M.N., F.B., S.C., and S.D. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

Data deposition: The sequence reported in this paper has been deposited in the European Molecular Biology Laboratory database (accession nos. FN393058–FN393060, FN393062–FN393087, FN394216, and FN394217).

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This article contains supporting information online at www.pnas.org/cgi/content/full/0904673106/DCSupplemental.

(303 ORFs), in-frame stop codons (25 ORFs), or the absence of start or stop codons due to the presence of SNPs or indels (15 ORFs). We identified 11 Ty elements in the assembly (2 Ty1, 7 Ty2, 1 Ty4, and 1 Ty5), whereas 50 such elements have been identified in the S288c genome. We detected no Ty3 elements. This depletion of Ty elements is consistent with the results of comparative genome hybridization for the EC1118 strain (12). This overall picture was further supported by a direct estimate of the overall Ty abundance from sequencing reads (1.8%), much lower than that in S288c (3.4%), which was found to have the highest Ty abundance in a previous population study (5). This analysis also confirmed a clear inversion in the proportions of Ty1 and Ty2 in EC1118 compared with S288c.

Genes Present in S288c but Missing from EC1118. In total, 111 of the genes present in S288c were not found in the EC1118 genome (Table S4). Most of these genes are repeated and located in subtelomeric regions, which have not been accurately assembled, making it difficult to estimate copy number precisely. However, several of these genes (e.g., *HXT16*, *PAU21*, and *SORI*) are known to vary in copy number between strains (7, 12, 14). A large 17-kb telomeric region on chromosome VI encompassing *YFL052W* to *YFL058W* was absent in EC1118. Nontelomeric genes (21 genes) were also found absent from EC1118. They consist mainly of genes that are present in tandem duplicated arrays (*ENA2/5*, *MST27*, *PRM8*, *ASP3*, and *FCY22*) or in a 20.5-kb region of chromosome XII adjacent to the rDNA array, including 4 copies of *ASP3*. Most of the missing nontelomeric genes were found frequently deleted in other *S. cerevisiae* strains (Table S4). Two missing genes, *MST27* and *PRM8*, belonging to the *DUP240* family, have been found depleted in other wine yeasts (12, 16).

Genes Present in EC1118 but Missing from S288c. We identified 34 ORFs in EC1118, encoding proteins of 50 to 150 aa, that were absent from S288c. Only 6 of these ORFs were kept in EC1118 annotation (Table S3), thanks to the presence of identified orthologs in *S. cerevisiae* strains YJM789, RM11-1a, and AWRI1631 and conserved genomic sequences in *Saccharomyces sensu stricto*: EC1118.1J19.0562g, present in most of these strains and species, EC1118.1G1.0023g, highly conserved in *S. mikatae*, the duplicated EC1118.1M36.0034g and EC1118.1M36.0045g, present in a single copy in *S. mikatae* and in AWRI1631 and in 2 copies in *Schizosaccharomyces japonicus*, and two other genes with a defined function. The first gene, *KHR1* (EC1118.1I12.1684g), which encodes a heat-resistant killer toxin, is located in a 1.6-kb fragment inserted into EC1118 chromosome IX and flanked by 2 LTR elements. *KHR1* was also found at the same location in the genome of YJM789. The second gene, EC1118.1O30.0012g, is predicted to encode Mpr1, a protein with N-acetyltransferase activity conferring resistance to oxidative stress and ethanol tolerance (17). This ORF has been identified in the Σ 1278b strain and, interestingly, also in other wine yeasts (RM11-1a and AWRI1631).

We also found another 34 genes and 5 pseudogenes to be present in EC1118 but missing from S288c. Unlike the genes described above, these genes were organized into 3 large clusters that have been analyzed in detail (see below).

Identification and Localization of Large Chromosomal Regions Unique to EC1118. Three large regions of the EC1118 genome, a total of 120 kb in length, which could not be aligned with the S288c reference genome, were identified (Fig. 1).

The first of these regions was 38 kb long (region A) and was located in the subtelomeric region of the left arm of chromosome VI. The extremity of this chromosome displays a high degree of rearrangement (Fig. 1). A 23-kb fragment in the left arm of chromosome VI (including *YFL052W* to *YFL062W* in S288c) is

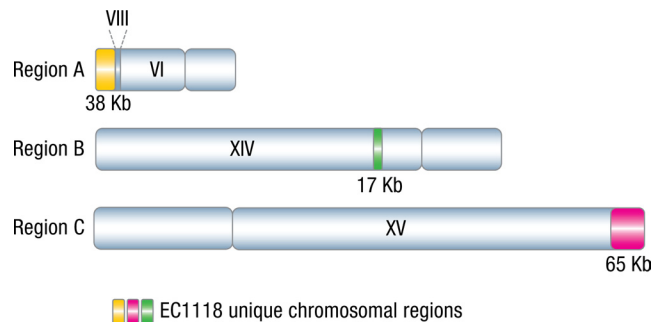


Fig. 1. Chromosomal distribution of the 3 unique EC1118 regions. The alignment of EC1118 contigs with S288c chromosomes led to the identification of 3 genomic regions unique to EC1118. The localization and length of these 3 regions are indicated by colored chromosomal segments. The insertion into chromosome VI of a 12-kb fragment from chromosome VIII is also shown.

absent. An internal part of this region encompassing the genes from *YFL059W* to *YFL062W* (5 kb) was found inserted into the right telomeric end of chromosome X. Second, a 12-kb fragment originating from chromosome VIII (including *YHR211W* to *YHR217C*) was found in the 3' region of *YFL051C* (Fig. 1) resulting in *YFL051C* being fused to *YHR211W* (gene EC1118.1F14.0155g). The sequences of *YFL051C* and of *YHR211W* are highly similar, suggesting that the translocation was mediated by homologous recombination. Similar translocations to chromosome X were also found in strains YJM789 and RM11-1a. PCR, sequencing, and Southern blot analysis on EC1118 chromosomes confirmed these rearrangements.

We identified a second unique region (region B) as a 17-kb insertion into chromosome XIV, between genes *YNL037C* and *YNL038W*. Interestingly, a sequence similar to region B was detected in the RM11-1a genome, but the sequence is slightly rearranged compared with EC1118 and located between genes *YNL248C* and *YNL249C*. We confirmed the localization of region B in EC1118 by PCR amplification of the breakpoints.

A third region, 65 kb in length (region C), was identified in the subtelomeric region of the right arm of chromosome XV, replacing the last 9.7 kb of this chromosome. Southern blot analysis confirmed the location of region C on chromosome XV.

Function of the EC1118 ORFs Encompassed by the Unique Regions. Within the three unique EC1118 regions, 34 ORFs predicted to code for proteins of >150 aa in length and with homologs in other species were identified (Table S5). These genes were classified according to the Munich Information Center for Protein Sequences (MIPS) functional catalog and were found to be involved mostly in key functions of the winemaking process, such as carbon and nitrogen metabolism, cellular transport, and the stress response (Fig. 2).

During wine fermentation, yeast cells must convert large amounts of glucose and fructose into alcohol. This process is also limited by nitrogen. Twenty of the 34 newly identified genes were found to encode proteins potentially involved in the metabolism and transport of sugar or nitrogen. These genes included genes similar to those encoding a *Kluyveromyces thermotolerans* glucose transporter, the *S. cerevisiae* glucose high-affinity transporter *HXT13*, and the *S. pastorianus*-specific fructose symporter *FSY1*. Several of these genes have homologs with known functions in amino acid metabolism, such as a transcription factor involved in proline utilization (*PUT3*), a *S. cerevisiae* permease potentially involved in the export of ammonia (*ATO3*), and 2 tandem-repeated genes encoding permeases of neutral amino acids. Another example of genes encoding proteins with nitrogen-related functions is provided by the gene encoding 5-oxo-L-

in France, and from the Centraalbureau voor Schimmelcultures in the Netherlands. Cells were routinely grown in YPD medium (1% yeast extract, 1% peptone, and 1% glucose) at 28 °C, with shaking.

Gene Prediction and Annotation. Genome annotation was based on a combination of methods including ORF calling (minimum size, 150 bp), gene prediction with GlimmerHMM (43), and direct mapping of 5288c ORFs from the *Saccharomyces* Genome Database. The detailed annotation procedure and a complete annotation file are available in *SI Materials and Methods* and [Table S3](#).

Microsatellite Analysis. The 120 strains were characterized for allelic variation at 11 microsatellite loci, as described by Legras et al. (4). The chord distance *D_c* between strains was calculated, as described by Cavalli-Sforza and Edwards (44). The neighbor-joining tree was constructed with the PHYLIP 3.67 package (45) and drawn with MEGA software version 4.0 (46). The tree was rooted by the midpoint method.

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Additional Materials and Methods. Further details are available in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank Lallemand Inc. for providing the strain EC1118, Noemie Jacques for providing strains from the Centre International de Ressources Microbiennes–Levures, and Gianni Liti and Justin Fay for providing *S. cerevisiae* strains; the Gènelevures Consortium for providing free access to the genome of *Pichia sorbitophila*; Claude Gaillardin for helpful discussions and for providing Lebanese strains; Philippe Abbal for bioinformatics assistance; and Jean-Jacques Gonod for drawing figures. This work was supported by grants from the Consortium National de Recherche en Génomique-Genoscope (no. 2005/74 to S.D.) and the Bureau des Ressources Génétiques (no. 347 “Exploration de la diversité fongique” to S.C.). M.N. and E.B. hold postdoctoral fellowships from the Generalitat de Catalunya and Institut National de la Recherche Agronomique (INRA), respectively. This work was supported by INRA and Centre National de la Recherche Scientifique.