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Genomics update

Wine Genomics

Roland J. Siezen

Kluyver Centre for Genomics of Industrial Fermentation; TI Food and Nutrition, 6700AN Wageningen, The Netherlands; NIZO food research, 6710BA Ede, The Netherlands

What better way is there to celebrate the start of a new journal than with a glass of good wine and news of recent developments in wine genomics?

Winemaking is a complex process, in which many factors play a role in development of aromas responsible for sensorial wine properties, such as type of grape variety, berry development and ripening, as well as alcoholic fermentation by yeasts and malolactic fermentation by lactic acid bacteria. In this process, use of genomics tools is now rapidly leading to insights in molecular mechanisms of aroma and flavour development, stress responses and pathogen resistance. This will hopefully lead to even more new and exciting wines for us to enjoy!

Grape genomics and diversity

Grapes and their derived products have a huge worldwide market. The cultivated grape species *Vitis vinifera* has the potential to become a model for fruit tree genetics and genomics (Figure 1). All grapevine varieties are highly heterozygous, with as much as 13% sequence divergence between alleles, posing a major challenge to modern whole-genome shotgun sequencing. Two recent papers (Jaillon *et al.*, 2007; Velasco *et al.*, 2007) describe high-quality draft genome sequences of different cultivated clones of *V. vinifera* Pinot Noir, the grape used for the production of red and sparkling wines. Genome sizes were estimated at about 500 Mb, assembled into over 2000 metacontigs, most of which were anchored to 19 linkage groups (Table 1). About 30 000 genes are predicted in their genomes, and millions of single-nucleotide polymorphisms (SNPs) were identified. An extensive collection of ESTs (>300 000 sequences) is also available at the NCBI EST database (NCBI dbest [<http://www.ncbi.nlm.nih.gov/dbEST>]). Com-

pared to other sequenced plant genomes (*Arabidopsis*, rice, poplar), the draft grape genomes show a large expansion of enzyme families for biosynthesis of tannins, flavonoids and stilbenes – such as the antioxidant resveratrol associated with health benefits of moderate consumption of red wine – and of terpenoids which contribute to wine aroma volatiles (Figure 2). Typical terpenoids of aroma-rich grape varieties are linalool, geraniol, nerol, citronellol and α -terpineol. Over 2000 genes for transcription factors were found, many of which could be assigned to regulation of features such as accumulation of secondary metabolites, fruit ripening, anthocyanin and flavonols biosynthesis, ethylene signaling and defense responses.

Genetic diversity was further analyzed in 11 grape genotypes, selected to represent existing genetic variation, by combining a re-sequencing approach and SNPlex™ technology (Lijavetzky *et al.*, 2007). Sequencing of 230 gene fragments, representing over 1 Mb of grape DNA sequence, allowed the discovery of ~1700 SNPs with an average of 60 bp/SNP (43 bp/SNP in non-coding regions and 67 bp/SNP in coding regions). Nucleotide diversity in grape was found to be similar to values observed in highly polymorphic plant species such as maize. The value of these SNPs is that they can be used as molecular markers for linkage mapping, cultivar identification and genetic diversity studies.

Grape functional genomics

Affymetrix *Vitis* GeneChips^R (containing about 14,500 *V. vinifera* unigene probes) have been used in several studies of grape berry development to analyze differential gene expression in various tissues (skin, pulp and seed) of *V. vinifera* Cabernet Sauvignon (Grimplet *et al.*, 2007) and in distinct ripening phases of *V. vinifera* Pinot Noir (Pilati *et al.*, 2007) and Cabernet Sauvignon (Deluc *et al.*, 2007). Grape berry development is divided into three major phases on the basis of chemical and morphological traits: (I) initial berry growth, characterized by accumulation of organic acids (mainly malate and tartrate), (II) veraison, when berries start to change colour and soften, and (III) ripening, characterized by accumulation of sugars, pigments and flavour compounds. Both metabolite and mRNA expression profiling were performed in

Email: r.siezen@cmbi.ru.nl

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Fig. 1. Pinot Noir grapes and grapevine. Adapted from jim@jenkinswinery.com.

parallel to understand the transcriptional regulatory processes that ultimately influence the organoleptic properties of wine (Deluc *et al.*, 2007). Proteomics has been used to identify differences between *V. vinifera* Chardonnay and Cabernet Sauvignon and their responses to water deficit and salinity (Vincent *et al.*, 2007). These detailed surveys revealed the expression patterns for genes and pathways that play key functional roles in cell growth, in phytohormone biosynthesis and response, in transport and signaling, in metabolism of flavonoids, organic acids, amino acid, sugars and starch, and in berry ripening and softening.

Wine microbial consortia

After grape crushing, the juice (also known as *must*) can be left to ferment by the natural flora present on the grape

or in the winery but more commonly it is inoculated with commercial, selected yeast strains. Using PCR-DGGE and population enrichment, an extensive inventory has recently been made of the complexity and diversity of wine microbial consortia in six different red and white wine grape varieties at different stages of the winemaking process: on grape berries, in must during fermentation, in bottled wine and on vat and barrel surfaces (Renouf *et al.*, 2007). Diversity was greatest on grape surfaces, with 52 yeast species and 40 bacteria identified, and was dramatically reduced during winemaking and aging. The most common enological yeasts (*Saccharomyces cerevisiae*, *Brettanomyces bruxellensis*) and bacteria (*Oenococcus oeni*, *Pediococcus parvulus*, *Gluconobacter oxydans*) were present on grape skins from the first stages of development. Most resistant to alcoholic fermentation and aging were *S. cerevisiae*, *B. bruxellensis*, *O. oeni* and *P. parvulus*, being found throughout all barrel aging and after several years of bottling. The species most detrimental to wine quality, *P. parvulus* (produces histamine and ropiness) and *B. bruxellensis* (confers off-odours) can be easily identified and monitored using this procedure. Much of the autochthonous wine yeast biodiversity found on grape berries actually appears to derive from commercial yeasts that have been used as fermentation starters in the wineries in previous years (Schuller and Casal, 2007; Schuller *et al.*, 2007; Valero *et al.*, 2007).

Wine bacterial genomics

The primary fermentation by *S. cerevisiae* produces ethanol and creates the anaerobic environment that limits growth of other yeasts and bacteria, and thus protects the wine from spoilage. Malolactic fermentation (MLF) is the secondary fermentation that typically occurs after alcoholic fermentation and is carried out by one or more lactic acid bacteria. *Oenococcus oeni* is most commonly responsible for MLF in wine, converting malate to lactate and CO₂. MLF is thought to generally enhance the body and flavour persistence of wine, producing wines of greater palate softness and roundness. Due to these favourable attributes, *O. oeni* is frequently used as a

Table 1. Genome sequence projects relevant to wine making.

Species and strain	organism	Size (Mb)	ORFs	GC%	chromosomes	reference
<i>Vitis vinifera</i> PN40024	grapevine	487.1	30,434		38	(Jaillon <i>et al.</i> , 2007)
<i>Vitis vinifera</i> ENTAV115	grapevine	504.6	29,585		38	(Velasco <i>et al.</i> , 2007)
<i>Saccharomyces cerevisiae</i> S288C	yeast, laboratory	12.1	5,860	38.1	16	(Goffeau <i>et al.</i> , 1996)
<i>Saccharomyces cerevisiae</i> , various	yeast, wine	incomplete				Sanger Institute#
<i>Oenococcus oeni</i> MCW PSU-1	lactic acid bacterium	1.8	1,691	37.9	1	(Mills <i>et al.</i> , 2005)
<i>Oenococcus oeni</i> ATCC BAA-1163	lactic acid bacterium	1.8, incomplete	1,398	37.9	1	NCBI, acc. code NZ_AAUV00000000
<i>Gluconobacter oxydans</i> 621H	acetic acid bacterium	2.7	2,432	61.1	1	(Prust <i>et al.</i> , 2005)

Saccharomyces Genome Resequencing Project at the Sanger Institute (<http://www.sanger.ac.uk>) includes incomplete sequences of 37 *S. cerevisiae* strains, of which 7 are wine yeasts.

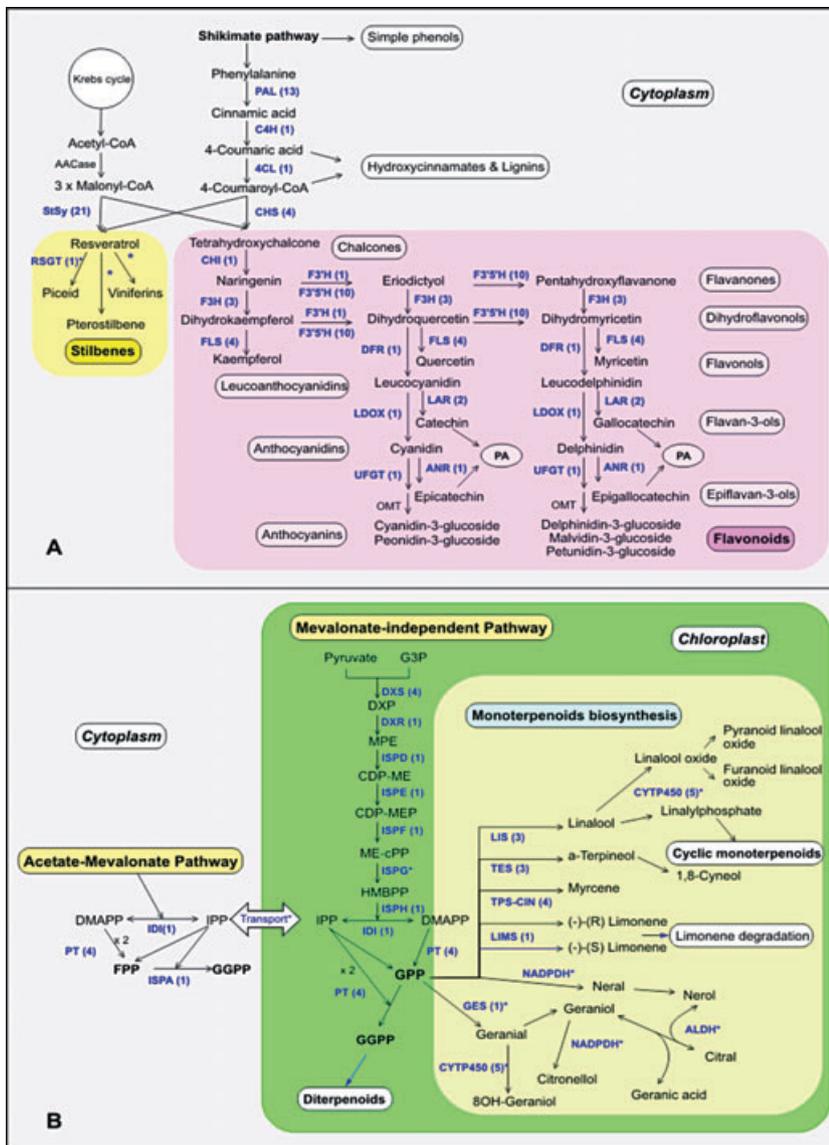


Fig. 2. *Vitis vinifera* pathways for phenolic and terpenoid biosynthesis. Reprinted from (Velasco *et al.*, 2007). See original paper for details of compounds, enzymes and pathways.

starter culture to promote malolactic fermentation. The genome sequence of *O. oeni* PSU-1 (isolated from a spontaneous MLF in wine) has been determined (Mills *et al.*, 2005; Makarova *et al.*, 2006; Makarova and Koonin, 2007). Genes related to flavour modification in wine, such as malolactic fermentation capacity and citrate utilization were identified, and consistent with its classification as an obligately heterofermentative lactic acid bacterium the *O. oeni* genome encodes all the enzymes for the phosphoketolase pathway. Diversity of wine *O. oeni* strains has recently been assessed using different genomics techniques such as comparative genomic analysis by subtractive hybridization (Delaherche *et al.*, 2006), multiplex random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) of selected markers (Renouf *et al.*, 2008), and a combination of RAPD-PCR, restriction endonucleases analysis-pulsed field gel electrophore-

sis (REA-PFGE) and differential display PCR (DD-PCR) (Lechiancole *et al.*, 2006). A large diversity was observed, and a relationship between the phenotypic and genotypic characterization of *O. oeni* strains isolated from wines with different levels of enological potential was shown. An applied outcome of these studies will be a better discrimination of starter cultures with regard to MLF performance and /or production of off-flavours.

Wine yeast genomics and diversity

Most wine fermentations these days begin by inoculating grape must with a specific yeast culture, which has the advantage of control and standardization of winemaking. Numerous features of both wine and the production process are dependent on the choice of yeast strains. Selection of yeast starters depends on the grape variety,

Table 2. A selection of wine flavour compounds that display yeast strain-dependent variation in concentration.

Compound		Sensory attribute
Volatile acids	Acetic acid	Vinegar, pungent
Higher alcohols	Isoamyl alcohol	Marzipan
	Phenylethyl alcohol	Floral, rose
Esters	Isoamyl acetate	Banana, pear
	Ethyl hexanoate	Apple, banana, violets
	Ethyl octanoate	Pineapple, pear
Carbonyl compounds	Acetaldehyde	Sour, green apple
Volatile phenols	4-Vinylguaiacol	Smoky, vanilla, clove-like
Sulfur compounds	Hydrogen sulfide	Rotten eggs
Volatile thiols	4-Mercapto-4-methylpentan-2-one	Cat urine, blackcurrant, broom

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must composition, fermentation conditions and the requirements of final products. Features such as fermentation performance (e.g. stress tolerance, nutrient utilization), down-stream processing (e.g. clarification, flocculation, sedimentation), alcohol content, and levels of desirable (e.g. resveratrol) or undesirable chemical compounds are all dependant upon the yeast strain used. Most important for consumer choice are the organoleptic properties of wine such as appearance, mouth-feel, bouquet, flavour and taste. These are determined by hundreds of metabolites (Table 2), and their balance can be significantly modified by the choice of wine yeast (Figure 3). The laboratory strain *S. cerevisiae* S288C was sequenced and published in 1996 (Goffeau *et al.*, 1996) and the most recent updated annotation can be found in

the Saccharomyces Genome Database (SGD; www.yeastgenome.org) (Fisk *et al.*, 2006; Hirschman *et al.*, 2006). In the Saccharomyces Genome Resequencing Project at the Sanger Institute (<http://www.sanger.ac.uk>), haploids of 37 *S. cerevisiae* strains, including 7 wine yeasts, have been sequenced to a coverage of 1x – 3x. The sequence data have been aligned to the reference genome sequence to identify all putative SNPs.

The growth conditions in an actual wine fermentation are very different from those found in laboratory conditions. For instance, laboratory yeast strains cannot transform all the sugar in grape must into ethanol under the conditions which occur in winemaking. Complete utilization of sugar is necessary to prevent subsequent growth of acetic and lactic acid bacteria on these residual sugars

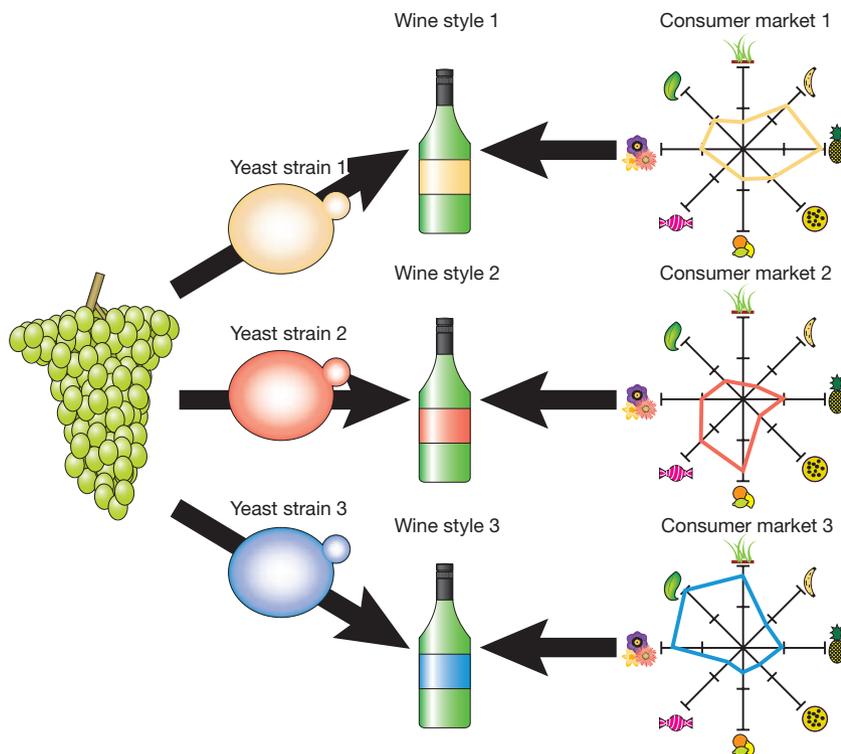


Fig. 3. Tailoring wine to match consumer preference. Owing to discrete differences in metabolism across strains of *S. cerevisiae* (shown as yeast strains 1, 2 and 3), wines that display very different sensory properties (wine styles 1, 2 and 3) can be produced by fermentation of identical grapes. These diverse wine styles will appeal differently to different consumer markets (e.g. consumer market 1 prefers wines with enhanced fruity flavours and aromas, while consumer market 2 values citrus and confectionary characteristics, and consumer market 3 herb and vegetable characteristics). Reprinted from (Borneman *et al.*, 2007), Copyright 2007 with permission from Elsevier.

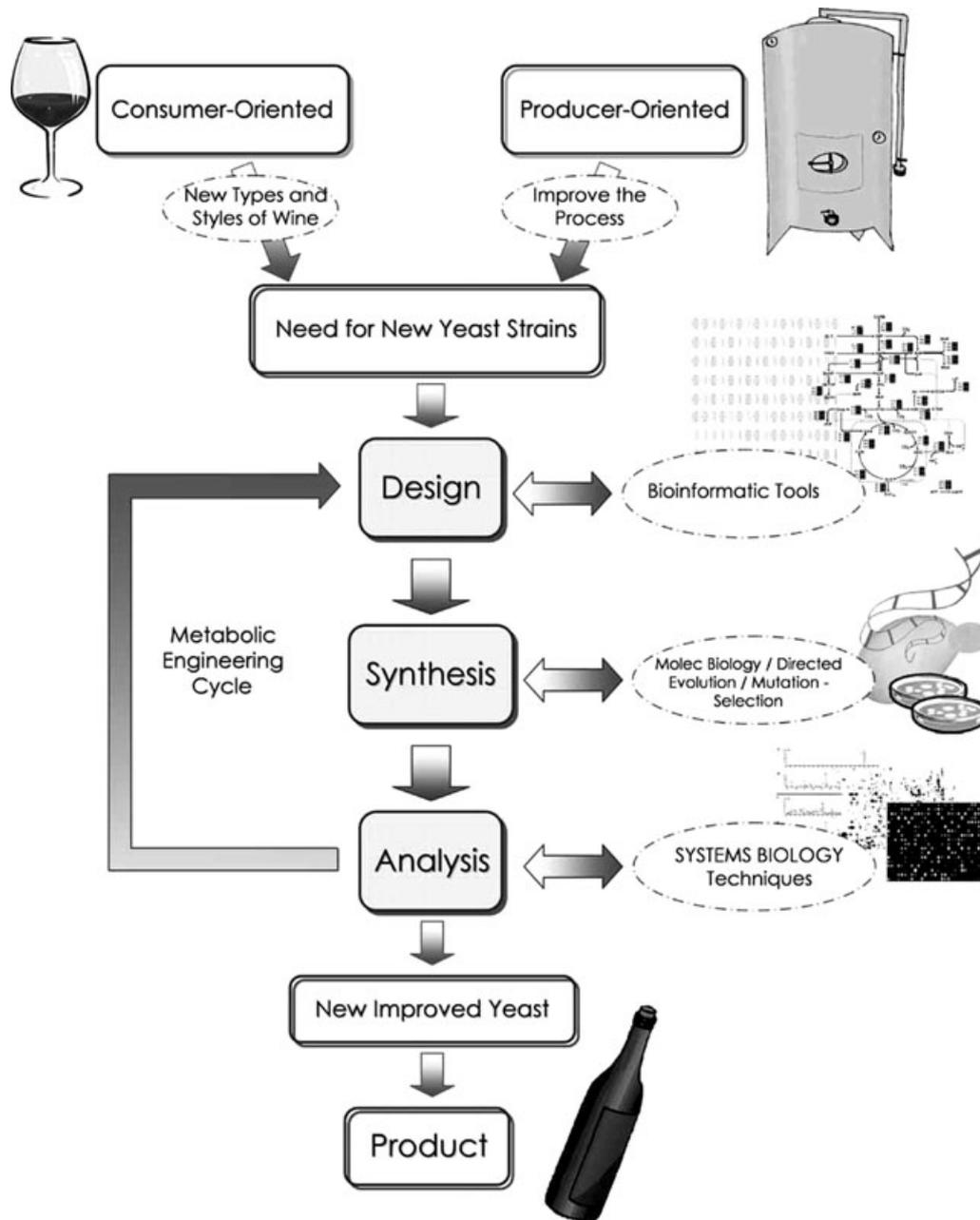


Fig. 4. Framework for the integration of genomic information, bioinformatics tools and molecular biology for the rational design of improved wine yeast strains.

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which can generate an increase in acidity and formation of off-flavours. Industrial wine yeast strains also have many distinctive features that allow them to adapt to industrial conditions where multiple stresses occur during fermentation, such as low pH, high osmolarity, high SO₂ content, nutrient limitation, temperature variations and ethanol toxicity. The many applications of post-genomics techniques to characterize and compare laboratory and industrial wine yeasts and unravel their diversity have been recently

reviewed (Bisson *et al.*, 2007; Borneman *et al.*, 2007; Pizarro *et al.*, 2007). This comparative analysis of laboratory and wine isolates is refining our understanding of the mechanisms of *S. cerevisiae* genome evolution. The strength of genomic analysis of *Saccharomyces* in native environments is in providing evidence for functions to previously uncharacterized genes and delineating the physiological parameters of ecological niche specialization and stress adaptation.

Wine yeast systems biology and strain improvement

The emerging field of systems biology aims to integrate computational and experimental genomics data, such that mathematical models of complex higher-order systems can be developed. Application of systems biology in the winemaking field has the exciting potential of further developing the understanding how differences arise in fermentation and wine flavour between various yeast strains, and how these characteristics could be modulated to tailor wine composition (Borneman *et al.*, 2007; Pizarro *et al.*, 2007). Winemakers seek to improve strain characteristics such as fermentation performance, stress tolerance, nitrogen assimilation and resistance to antimicrobials, and they strive to improve the biological control of spoilage microorganisms. Wine characteristics that could be further modulated are health and organoleptic properties, including lower concentrations of toxic compounds such as biogenic amines or ethyl carbamate, and lower alcohol content. While systems biology approaches to the understanding of the laboratory strain of *S. cerevisiae* have already led to tremendous advances in metabolic and cell engineering (Borneman *et al.*, 2007; Nielsen and Jewett, 2007) the implementation of this strategy in wine genomics will be dependent on the characterization and comparison of multiple genomes of industrial yeasts. With the advent of new and cheaper high-throughput DNA sequencing technologies, that feat will be realized in the not-too-distant future.

The wealth of genomics information being generated will soon be ready to be utilized for the selection and construction of strains with more desirable phenotypes, traits that will be designed to be genetically stable under commercial production conditions (Figure 4). Matching specific wines to consumer preferences delivers consumer satisfaction, while winemakers can maximise their economic returns. So let us raise our glasses and toast those whose mission is to develop wines for the future and to understand those of the past.

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