Comparative genomics in the wine bacterium *Oenococcus oeni*

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The production of wine from grape juice relies on the combined actions of both yeast and bacteria which shape the aroma and flavour of wine through the production of secondary metabolites and the biochemical transformation of many grape-derived constituents. Whereas the principal wine yeast, *Saccharomyces cerevisiae,* is primarily involved in the alcoholic fermentation in which glucose and fructose are converted into alcohol, the wine bacterium, *Oenococcus oeni,* is primarily involved in a secondary fermentation reaction where malic acid is decarboxlyated into lactic acid. This conversion, known as malolactic fermentation (MLF), results in an increase in wine pH and reduction in the sourness of the wine, while also providing microbial stability through the reduction of potential carbon sources for wine spoilage bacteria such as *Lactobacilli* and *Pediococci*[1]. In addition to its primary role in performing MLF, the metabolic by-products produced during the growth of *O. oeni in wine have been shown to positively contribute to the flavour and mouth feel of wines which have undergone MLF*[1].

Both the history and ecology of *O. oeni* are interesting. Despite wine bacteria and the malic acid degradation pathway having been identified as early as the mid-nineteenth century, *O. oeni* was not formally classified until the 1960s when it was originally named *Leuconostoc oenos* and thereby recognising this species as a member of the lactic acid bacteria (LAB)[2]. This classification was ultimately changed on the basis of molecular phylogenetic data to *O. oeni*, thereby forming a completely new genus of LAB with *O. oeni* as its sole member[3]. Even now, despite a renaissance in the identification of bacteria through the application of next-generation genome sequencing and metagenomics, there is only one other species of *Oenococcus*, *O. kitaharae* which has been identified to date[4]. Ecologically, despite *O. oeni* being readily isolated from wine, it has not been possible to find an 'environmental' reservoir of this species outside of wine and fermenting grape must[1,5,6]. Due to the highly seasonal nature of wine production, it remains a mystery as to how this organism is able to rapidly appear in significant numbers in finished wine to undertake the malolactic fermentation.

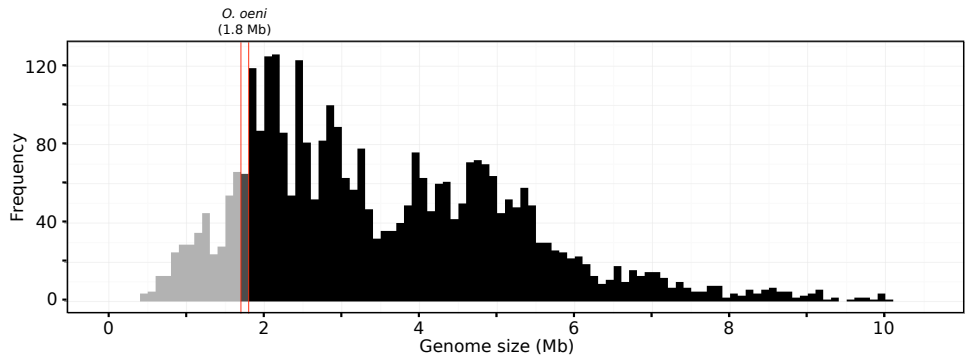

Figure 1. The distribution of bacterial genome sizes. Genome sizes were obtained for bacterial genome projects lodged with NCBI (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi). The frequency of individual genome sizes were then calculated based on a 0.1 Mb size bin with those greater than 10 Mb pooled into a single bin. The location of the *O. oeni* genome is indicated.

Given the potential economic benefits that the study of *O. oeni* could provide through the development of improved strains for wine production, this species has received significant research interest. However, the general intractability of *O. oeni* for many classical bacterial genetic techniques such as transformation, conjugation and transduction, provides a barrier to determining the molecular basis of desirable phenotypic traits in individual strains. In order to overcome these shortcomings, recent investigations into *O. oeni* have used comparative genomics techniques to categorise genetic diversity in this species[7,8]. The first genome sequence of *O. oeni*, strain PSU-1, was published in 2005 as part of a broad phylogenetic sequencing project which focused on the LAB group[8]. This showed that the genome of *O. oeni* was only 1.8 Mb and encoded about 1800 ORFs. This represents a streamlined genome, especially for a 'free-living' bacterium which is within the lower 10% of all bacterial genome sizes and at the bottom-end of what is observed in other species of LAB (Figure 1). This reduced genome size is at least partly due to the relatively predictable nutrient profile of wine, which contains significant amounts of many amino acids, carbohydrates (especially arabinose and xylose which are not utilised by wine yeast) and vitamins[9,10]. As such, *O. oeni* has been able to dispense with biosynthetic pathways such as those for many amino acids (strain PSU-1 appears to have the ability to biosynthesise only eight amino acids[11]), thereby reducing its overall genome size at the expense of reducing its potential environmental range.

This sequencing effort also paved the way for further investigations into the genetic diversity of *O. oeni* through the use of microarray-based comparative genome hybridisation (aCGH) analysis of genetic variation[7]. This investigation of 10 commercial *O. oeni* strains uncovered several large deletions that were present in many strains of *O. oeni* with some individual deletions accounting for a loss of over 1% of the *O. oeni* genome each (Figure 2). The genetic variation indicated by the aCGH analysis was subsequently corroborated through the sequencing of an additional two strains of *O. oeni*, ATTC BAA-1163 and AWRIB429[7]. In addition to the deletions which were indicated from the aCGH work, whole genome comparisons of the three strains showed significant variation which was also present in the form of single nucleotide polymorphisms (SNPs) and large insertions. Interestingly, it was also observed for at least one of the large deletions, that this area also coincided with genomic insertions such that at least one large, strain-specific cassette was observed in each of the three strains.

Work is now under way to expand on the genomic information which is currently available for *O. oeni*. Genome sequences for at least another 13 strains are under way and expected to be

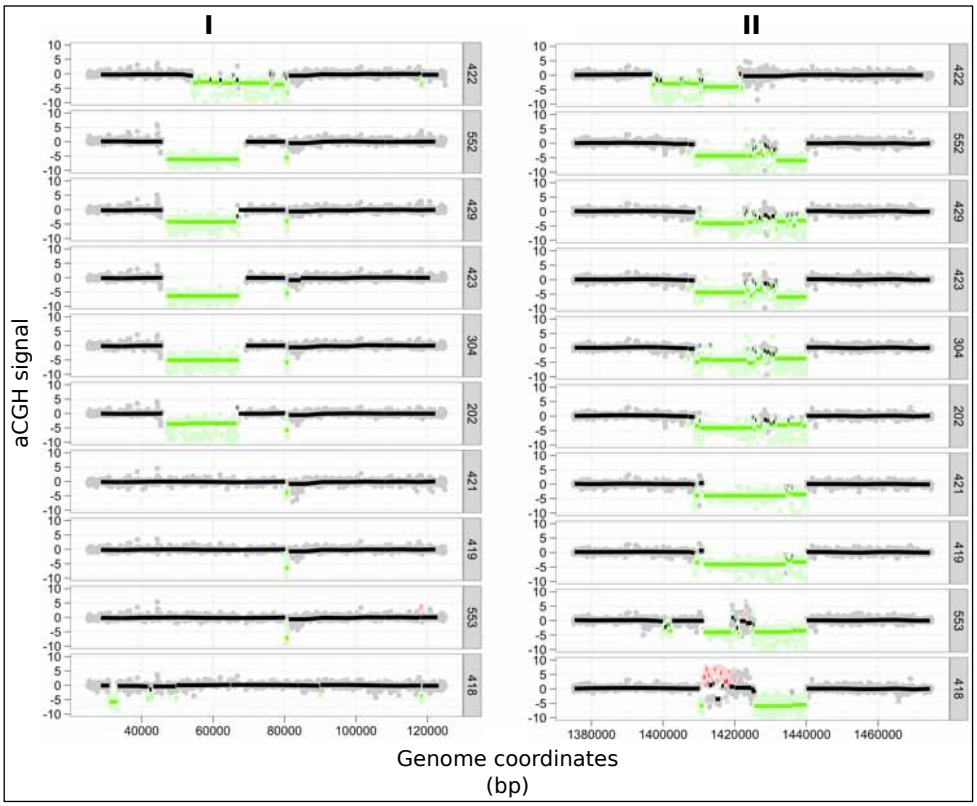

Figure 2. Large deletions present in the *O. oeni* genome. Microarray-based comparative genome hybridisation (aCGH) results for 10 strains of *O. oeni*. Microarray signals are relative to the genomic reference strain PSU-1. Green areas represent genomic deletions while red areas represent genomic loci with increased copy number relative to the reference. Two major deletions were observed at high frequency across the strains (I and II).

released soon and genome sequencing of *O. kitaharae* is also near completion (Borneman *et al*. unpublished). Systems biology experiments are also planned to attempt to link genetic variation with phenotypic data in the absence of molecular techniques. These data will provide a rich insight into the variation which exists within this enigmatic genus and how genetic variation within individual strains of *O. oeni* translates into important industrial phenotypes.

References

1. Bartowsky, E.J. (2005) *Oenococcus oeni* and malolactic fermentation – moving into the molecular arena. *Aust. J. Grape Wine Res.* 11, 174–187.
2. Garvie, E.I. (1967) *Leuconostoc oenos* sp. nov. *J. Gen. Microbiol.* 48, 431–438.
3. Dicks, L.M. *et al*. (1995) Proposal to reclassify *Leuconostoc oenos* as *Oenococcus oeni* [corrig.] gen. nov., comb. nov. *Int. J. Sys. Bacteriol.* 45, 395–397.
4. Endo, A. and Okada, S. (2006) *Oenococcus kitaharae* sp. nov., a non-acidophilic and non-malolactic-fermenting oenococcus isolated from a composting distilled shochu residue. *Int. J. Sys. Evol. Microbiol.* 56, 2345–2348.
5. Renouf, V. *et al*. (2005) Understanding the microbial ecosystem on the grape berry surface through numeration and identification of yeast and bacteria. *Aust. J. Grape Wine Res.* 11, 316–327.
6. Wibowo, D. *et al*. (1985) Occurrence and growth of lactic-acid bacteria in wine – a review. *Am. J. Enol. Vitic.* 36, 302–313.
7. Borneman, A.R. *et al*. (2010) Genotypic diversity in *Oenococcus oeni* by high-density microarray comparative genome hybridization and whole genome sequencing. *App. Microbiol. Biotech.* 86, 681–691.
8. Makarova, K. *et al*. (2006) Comparative genomics of the lactic acid bacteria. *Proc. Natl. Acad. Sci. USA* 103, 15611–15616.
9. Hernandez-Orte, P. *et al*. (2002) Relationship between varietal amino acid profile of grapes and wine aromatic composition. Experiments with model solutions and chemometric study. *J. Agric. Food Chem.* 50, 2891–2899.
10. Henschke, P.A. and Jiranek, V. Yeasts – metabolism of nitrogen compounds. In Wine Microbiology and Biotechnology, GH Fleet (Ed.), Harwood Academic Publishers, Chur, Switzerland, 1993, pp. 77–164.
11. Mills, D.A. *et al*. (2005) Genomic analysis of *Oenococcus oeni* PSU-1 and its relevance to winemaking. *FEMS Micro. Rev.* 29, 465–475.

Biographies

Anthony Borneman obtained his PhD in 2002 from the Genetics department at the University of Melbourne with Prof. Michael Hynes and Dr. Alex Andrianopoulos where he studied the regulation of morphology in the fungal pathogen *Penicillium marneffei*.

Anthony then spent four years as a postdoctoral associate with Prof. Michael Snyder at Yale University where he applied whole genome techniques to compare transcriptional networks across several yeast species.

Anthony is currently working as a Senior Research Scientist at the Australian Wine Research Institute where he is using next-generation sequencing and comparative genomics to investigate the genetic basis of phenotypic diversity in industrial microorganisms such as the yeast *Saccharomyces cerevisiae* and malolactic bacterium *Oenococcus oeni*.

Eveline Bartowsky is a Senior Research Microbiologist at AWRI, and directs the malolactic fermentation (MLF) and wine bacteria research program, and is Manager of the AWRI Wine Microorganism Culture Collection. Her research interests include projects on aroma and flavour aspects of MLF; MLF inoculation regimes to improve MLF efficiency; genomics of Oenococcus oeni; and minimising wine spoilage by lactic acid and acetic acid bacteria. Eveline is world recognized for her research into wine bacteria and has published over 80 papers including journal articles, book chapters and technical papers.

Following a PhD in microbiology from The University of Adelaide, Eveline undertook postdoctoral studies in Sweden (Umeå University, Umeå), USA (Washington University Medical School, St. Louis) and University of Adelaide before joining the AWRI in 1994. She has wide experience with a diverse group of bacteria: *Vibrio cholerae*, *Citrobacter freundii*, and wine associated Lactic Acid Bacteria and Acetic Acid Bacteria.