Technical notes

Genomics in the study of microbial diversity, regionality and terroir

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DNA is the blueprint of life. It resides in every living organism, from bacteria to complex plants and animals and represents an encrypted repository of the thousands of instructions required for cells to grow and respond to their environment. Genomics is the science behind decoding and understanding these instructions. However, until very recently, widespread application of genomics was limited by the enormous time and cost required to obtain and translate the genomic information of even the simplest life forms, such as bacteria or yeast, into a useable form. The recent advent of next-generation DNA sequencing is now revolutionising genomics by eliminating these time and cost barriers and providing the impetus for its application in settings such as medicine and agriculture.

Winemaking is arguably the oldest biotechnology, dating back over 7000 years, and this industry has a strong history of applying cutting edge scientific research to improving quality and production (Borneman, Schmidt and Pretorius 2013). Given the roles of grapevines, yeast and bacteria in shaping the composition of finished wines, it is not surprising that genomics is poised to play an increasing role in unlocking the potential of these biological inputs. The most direct application of this will be in identifying and linking genetic variation in the specific grapes, yeast and bacteria that are present in the wine fermentation with the production of desirable (or the abatement of undesirable) winemaking characteristics.

One genomic technology that is poised to have an impact on agriculture, and especially wine production, is metagenomics. Metagenomics is a term that describes an extension of genomics (which normally focuses on determining the DNA sequence of a single species or strain at a time) to the sequencing of DNA isolated from environmental samples (e.g. water, soil, air) that are complex mixtures of microorganisms. The use of metagenomics has been highlighted by two large consortia, 'The human microbiome project' and 'The earth microbiome project' that have sought to determine the microbial composition of thousands of samples from various sites in and on the human body and from natural environments (Proctor 2011, Jansson and Prosser 2013). The ultimate aim of metagenomics is to enable the correlation of the presence

of specific microbial genomes (or metabolic pathways) with specific traits. However, due to the complexity of many microbial communities, true metagenomic sequencing cannot be achieved, even when using the most cutting-edge of current next-generation technologies. In many of these situations, a scaled-back form of metagenomics, often termed 'meta-barcoding' or 'phylotyping' can be employed to efficiently measure the proportions of microbial species present by using a small portion of the genome as a 'genomic barcode'.

Of the numerous potential applications of metagenomics in the wine industry, shining a light on the microbial influences on wine production and regional terroir are likely to be the most informative. In the vineyard, it is now not only possible, but economically feasible, to compare microbial populations from conventionally farmed, organic and biodynamic vineyards or similarly managed vineyards in different geographical locations. This type of work will provide firm scientific data regarding the effects of geography combined with different vineyard practices on the soil microbiota while also providing a means to assess the effects of viticultural interventions.

In the winery, metagenomics will find its most useful applications in tracking populations of yeast and bacteria during the winemaking process, and will be especially useful in teasing apart the drivers of individual uninoculated (wild) fermentations. In the absence of the high numbers of *S. cerevisiae* cells that are introduced through inoculation with active dried yeast, wild ferments show a prolonged, complex succession of diverse microbial species that only converge on *S. cerevisiae* as the fermentation nears its end (Fleet 2008). It is the varied metabolic contribution of the non-*Saccharomyces* yeasts at the early stages of wild fermentations that are thought to contribute to the complex characteristics of wild ferment wines.

The labour-intensive microbiological methods that have previously been used for analysing microbial communities provided limited data at relatively high cost, and therefore limited the application of these techniques outside small numbers of research projects. Applying metagenomic tools, such as barcoding, to the study of wild fermentation provides an economically efficient means to obtain highly detailed data on the composition of large numbers of samples. By leveraging the collective power of large sample numbers it will become possible for example, to correlate species composition of individual fermentations with final wine composition, or to judge the effect of geography or viticultural/winemaking intervention (harvest method, temperature, SO_2) on wine microbiota (and subsequent wine attributes). Initial application of this technology has proven useful for tracking bacterial and fungal composition during wine production and in relating geography and vineyard terroir (Bokulich and Mills 2013, Bokulich et al. 2014). AWRI Commercial Services has recently

launched a next-generation sequencing service that can identify the dominant yeast species present in wild ferments to enable Australian winemakers to access this powerful technology.

Winemaking is a microbiological process and the rapid development of genomic technologies, such as metagenomics, is poised to revolutionise all aspects of this ancient biotechnology. Embracing these new technologies will enable fresh insights into the biological inputs (grapevine, yeast, bacterial and human) of winemaking while helping to define Australian regional terroir, providing a means to improve the competitiveness of Australian wines on the world stage.

References

Bokulich, N.A., Mills, D.A. (2013) Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-high-throughput profiling of fungal communities. Appl. Environ. Microbiol. 79: 2519–26.

Bokulich, N.A., Thorngate, J.H., Richardson, P.M., Mills, D.A. (2014) Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. Proc. Nat. Acad. Sci. 111: E139–48.

Borneman, A.R., Schmidt, S.A., Pretorius, I.S. (2013) At the cutting-edge of grape and wine biotechnology. Trends Genet. TIG 29: 263–71.

Fleet, G.H. (2008) Wine yeasts for the future. FEMS Yeast Res. 8: 979–95.

Jansson, J.K., Prosser, J.I. (2013) Microbiology: The life beneath our feet. Nature 494: 40–1.

Proctor, L.M. (2011) The human microbiome project in 2011 and beyond. Cell Host Microbe 10: 287–91.

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