Developing culture independent analysis techniques and skills to analyse wild yeast fermentation in red and white wines





FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **GWT 1215**

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Date: **18/8/2013**

Abstract:
This travel was initiated with an outcome of travel grant GWT1116 after contact with Professor David Mills (UC Davis) in 2012. The purpose of this travel to UC Davis was to learn new techniques in microbial population analysis in wine through the application of next-generation sequencing. The knowledge gained in the preparation of samples for next-generation sequencing and the data analysis of the results will be used to analyse Australian fermentation samples.

Executive summary:

- Simon Dillon (PhD student-University of Adelaide) travelled to the laboratory of Professor Davis Mills (University of California Davis) to learn techniques in microbial population analysis.
- Techniques were observed from PhD student Mr Nick Bokulich in both sample preparation and data analysis.
- Microbial samples from 2013 vintage in Australia (current GWRDC funded PhD project (GWR Ph1108) were also analysed using the Illumina sequencing platform.
- Utilize the knowledge data from time spent in UC Davis to generate data for dissemination to the research and winemaking communities
- Travel to the Napa, Sonoma and Central Valleys of California to meet with practitioners of wild fermentation and conventional fermentation technologies.
- Workshop presentation at the 15th AWITC (workshop 13: 'The power of non-conventional yeast in fermentation')

Key activities and outcomes

The use of next-generation sequencing technologies has the power to unravel the complex interactions and species present in wild wine fermentations. At UC Davis the Mills laboratory has been using next-generation sequencing techniques for a number of years to perform community analysis on various types of alcoholic and non-alcoholic fermented foods (wine, beer, yogurt etc.). They are considered some of the world best practitioners the genetic analysis of fermented foods, and such was a key opportunity to learn from this group.

The PhD student in the Mills laboratory working with uninoculated (wild) fermentation was Mr Nick Bokulich who has a number of publications in the area of next-generation

techniques to analyse fermentation. The aim was to learn the basic protocols of the sample preparation including PCR of discrete DNA sequences that differentiate wine yeast and bacteria, bar-coding of DNA and the purification of DNA ready for analysis. The subsequent analysis was performed by the sequencing facility at UC Davis.
This was a culture-independent technique that analyses all DNA from the target organisms (in this case yeast and bacteria) by sequencing short amplicons of DNA that had been extracted directly from environmental samples (wine lees)

Samples taken from vintage 2012 in the Barossa Valley (current project GWR Ph1108) were taken as DNA extracts to the Mills laboratory and processed along with samples taken from Nick Bokulichs' work to reduce costs of sequencing. Samples from each day of a wild fermented and an inoculated Eden Valley Viognier (15 days) and a three time points in a wild fermented and an inoculated 2012 Barossa Shiraz.
Samples were transported to the laboratory as DNA extracts which then underwent PCR with specific primers used to amplify the rDNA ITS regions used in the differentiation of yeast and a separate PCR to amplify the V4 region of the bacterial genome so as to differentiate the bacteria present in the samples (including the lactic acid bacteria critical to winemaking). These samples were supplied to the sequencing facility at UC Davis and analysed using the Illumina Mi-Seq equipment. The data is currently being analysed here in Adelaide.

The second aim was to learn the analysis of the sequenced DNA via the QIIME analysis pipeline. The use of QIIME was preferred by the Mills laboratory for all microbial community sequencing analysis and uses a LINUX based series of algorithms to unravel the community structures from the DNA reads by using either online or in-house reference databases. This program can be utilised by anyone seeking to analyse data as it is an open source application. Mr Nick Bokulich is also a power-user of this program and has contributed to its ongoing software development.
As the data generated from the 2012 samples was unavailable at the time the use of QIIME with a practice data set and a data set used for a previous study was used for the training in the use of the software. The software suits analysing bacterial community data well, however due to the limits of the databases publically available the yeast data processing requires additional processing and is more time and resource intensive.

The use of this software and data analysis required the purchase and set up of a new computer (in Adelaide) for the data obtained in Davis. QIIME setup and data processing is underway with help from Nick Bokulich.

Travel to the largest wine regions in California was primarily to see the technologies used in the manufacture of wild fermented wines and to talk to the winemakers/oenologists involved. Wine production in Napa and Sonoma Valleys (and somewhat in the Central Valley) is different to Australian wine production due to lower labour costs and the location of highly populated urban centres close to the regions. The use of wild fermentation in the Napa and Sonoma Valleys is very common and most small to medium wineries bottle and market wild fermentation wine actively which is not similar to the situation in Australia. The use of inoculated yeasts is seen as an excellent fermenter but not for giving individuality or regional difference. A number of wineries were visited in this trip including Frogs Leap, Sterling Vineyards, Kaz Winery, Chalk Hill, Ridge Vineyards, Grgich Hills Estate and Ravenswood Winery. Speaking to winery staff and winemakers indicated respect in using wild fermentations and some even practiced is in preference to inoculated fermentation. The use of technology was however little different in wild fermentations as opposed to current vinification practices. The differences being that the majority use organic viticulture for wild fermented wine (due to marketing) and no or little use of SO_2 and filtration. Wines that were not sulfited or filtered before bottling weren't a concern for the winemakers in terms of future spoilage.

A workshop presentation at the 15th AWITC was completed along with 3 other speakers and 3 winemakers on the use of *Saccharomyces* hybrids and non-*Saccharomyces* yeast. Workshop 13 "The power of non-conventional yeast in fermentation" was attended by approximately 30 people on Sunday the 14th of July. The title of the presentation for this workshop was "Wild about wine" and presented data obtained from the current PhD project (GWR Ph1108). In addition 6 wines were presented as a part of the wine tasting session looking at the differences between wild and inoculated 2012 Viognier wines. Discussion regarding the outcomes of 'wild' fermentation in winemaking was held with the attendees. Interest was shown in conducting 'wild' fermentation and sequential non-*Saccharomyces*/*Saccharomyces* wine fermentations and learning the outcomes of the sequencing data analysis.