

A personal touch for wine microbiology

The Wine Microbiology and Microbial Biotechnology Laboratory at the University of Adelaide has developed a customized solution offering fully automated sampling for yeast fermentations. Based on a Freedom EVO® 200 platform, this system frees researchers from the need to manually aliquot samples day and night for up to three weeks.



Members of the Wine Microbiology and Microbial Biotechnology Laboratory team with the customized Freedom EVO

The Wine Microbiology and Microbial Biotechnology Laboratory is part of the University of Adelaide and the Wine Innovation Cluster. Led by Professor Vladimir Jiranek and Associate Professor Paul Grbin, the group aims to improve the winemaking process by developing new yeast strains offering characteristics such as faster fermentation rates, higher tolerance to ethanol and improved nutrient utilization. Dr Tommaso Liccioli, a postdoctoral research fellow in the laboratory, explained: “Winemaking is a complex process, with grape juice undergoing many biochemical and chemical reactions to become wine. These processes are largely carried out by yeast and bacteria, which have been ‘domesticated’ for thousands of years to withstand the harsh conditions during fermentation and impart specific

characteristics to the finished product. However, new wine styles, modern viticulture techniques and the effect of climate change are pushing fermentation conditions to extreme levels, challenging the fitness of these micro-organisms. This can result in problems during fermentation, leading to undesirable wine characteristics and financial losses for the industry.”

“Our aim is to develop novel yeast and bacteria strains which improve the winemaking process. However, because the use of genetic engineering techniques is prohibited in the food and beverage industry in most global markets, we focus on more traditional approaches, such as directed evolution and mutagenesis. These methods can be equally effective, but are often very time consuming, requiring large-scale screening of

hundreds or even thousands of strains, often from heterogeneous populations. Monitoring the fermentation process for large numbers of samples is also very laborious, requiring regular sampling of fermentation vessels every six, four, or even two hours over the course of several days to several weeks.”

“An important part of our job is to modernize these classical microbiological techniques and develop powerful new approaches for microbial improvement. We already had a number of robotic processing systems in the lab, as well as the necessary programming and engineering expertise in house, and so we were interested in developing a system that would allow fully automated screening. We looked at the various liquid handling platforms on the market, and Tecan’s Freedom EVO really stood out, enabling us to build a walkaway fermentation monitoring solution that would generate 96-well microplates ready for loading onto our various analytical instruments. The real strength of the Tecan system was its flexibility – including the software – which allowed us to integrate our own custom hardware solutions onto the platform with relative ease.”

“Our first task was to create a jacketed carrier system allowing 96 fermentations to run simultaneously on the Freedom EVO’s workdeck. We developed four 24-position jacketed carriers which have warm water pumped through them to maintain the optimal fermentation temperature, and each carrier has a 24-position stirrer underneath to continuously mix the fermentations. Each flask has also been



fitted with a custom-designed airlock along with a silicon septum in the top, allowing the workstation's Liquid Handling (LiHa) Arm to sample the anaerobic fermentations using fixed tips. At user-defined intervals, aliquots are transferred directly from the flasks to purpose-built cooled microplate carriers which maintain the samples at 10 °C until the user is ready to remove them from the workstation for analysis. It only takes half a day to set up a 96-fermentation experiment, then you can just remotely monitor its progress via a smartphone from another lab, from home or – because this is Australia – from the beach. Compared to having to be in the lab every few hours, day and night, this is fantastic. Everyone here loves the system, and is using it a lot.”

“Although we had previous experience with Tecan's equipment and services – we already had an Infinite® M200 microplate reader in the lab – we had no real way of knowing if this system was going to work, as we weren't aware of anyone that had tried anything similar. However, the Tecan team here in Australia was extremely good. Their communication was great, and they helped us with protocol development

and script writing. The platform's technical specifications were also very thorough in terms of exactly what the instrument could do, what pressures could be applied to the LiHa tips, etc., so we were fairly confident. Once we had designed and built our bespoke hardware and put everything together, it worked incredibly well first time. We got extremely good results straight away and, after a bit of fine tuning, we're very happy with the system.”

“At the moment, samples are still analyzed offline, but the next step is to develop a system that monitors at least a few key metabolites – such as sugar and alcohol concentrations – during the fermentation. One option is to integrate a small, benchtop NMR system underneath the workdeck of the Freedom EVO, and we are already discussing this with an instrument manufacturer in New Zealand. We could then run the NMR analyses in virtually real time, and still collect an aliquot of each sample in microplates to perform offline assays. It would also be good to replicate our set-up in other labs in Australia or further afield, allowing direct comparison of results between platforms.”

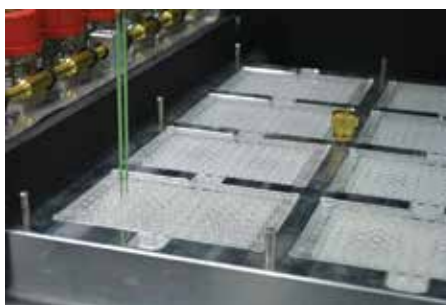
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To find out more about Tecan's food sciences solutions, visit www.tecan.com/food

To learn more about the Wine Microbiology and Microbial Biotechnology Laboratory, go to www.agwine.adelaide.edu.au/winemicro



The LiHa Arm's fixed tips allow direct transfer of samples from fermentation flasks...



...to cooled microplates, ready for offline analysis