Tomas Linder

Research fellowship subject title/theme number:

Mining orchard yeast diversity for novel horticultural biocontrol agents

Transformational technologies and innovation (theme III)

Host institution: Agriculture Victoria, Bundoora, Victoria, Australia

Host collaborator: Paul Cunningham

Dates of fellowship: August 1st 2022–May 31st 2023 (on location in Agriculture Victoria, Australia, November 2nd–December 15th 2022)

The author consents to this report being posted on the Co-operative Research Programme's website.

1. Objectives and importance of the research project

The initial project proposal was submitted in early autumn of 2019, which was then awarded in late 2019. Within the framework of the project, the author (yeast biologist affiliated with the Swedish University of Agricultural Sciences, Uppsala, Sweden) would travel to Agriculture Victoria (situated in Bundoora outside central Melbourne, Australia) for 13 weeks starting in late March 2020. The proposed research project at that time aimed at investigating how intra-species variation among insect-associated yeasts affect their attractiveness to their most common host insects through the secretion of volatile metabolites. The basic hypothesis under investigation was whether a yeast strain derived from the same locality as the host insect would be more attractive than a yeast strain of the same species derived from a locality further away (e.g. another continent). Such information may be of practical application when designing olfactory lures for pest insects, which often depend on yeasts as an important source of nutrients.

The initial project aimed to look at yeasts associated with two important horticultural pest insects: the Queensland fruit fly (*Bactrocera tryoni*) and *Carpophilus* sap beetles. The host group led by Associate professor Paul Cunningham had already demonstrated the importance of yeasts in lifecycle of both pests.

However, just as the project was about to commence, the global covid-19 pandemic started and the project was postponed. By the time covid-19 restrictions started to be lifted in Australia in mid-2022, the applicant had been promoted to senior lecturer, which made a consecutive 13-week stay in Australia difficult to reconcile with the new teaching responsibilities that came with the position. In consultation with the OECD Co-operative Research Programme (CRP), an amended project proposal was submitted and approved, which shortened the stay in Australia to six weeks (early November to mid-December 2022) with a remaining six weeks spent at the home university in Sweden in preparation for the trip (three weeks in August 2022) as well as three weeks dedicated to compiling and analysing data once the applicant had returned to Sweden (three weeks in spring 2023).

Since the research priorities of the host lab had also progressed somewhat since the initial proposal, the focus of the amended proposal switched from Queensland fruit fly and *Carpophilus* beetles to the small hive beetle (*Aethina tumida*). Small hive beetle larvae damage beehives by burrowing through the wax comb and consuming honey and pollen as well as live honey bee eggs, larvae and pupae. Larval defecation within the hive causes the honey ferment, making it unsuitable both for sale and bee food. If severe, a small hive beetle infestation of an individual hive may contribute to colony collapse.

The amended project would focus primarily on the yeast species *Kodamaea ohmeri*, which has a well-documented association with small hive beetle. As yeast lures are currently used for trapping small hive beetle in the field, the desire was to investigate whether geographic origin of *K. ohmeri* strain might affect its attractiveness. If so, it could be possible to tailor specific *K. ohmeri* strains to specific geographic localities for maximum trapping efficiency. It might also be possible to create synthetic blends of olfactory compounds that mimic the volatile profiles of the most attractive yeast strains.

2. Achievement of fellowship objectives

The main obstacle towards achieving the stated goals of the amended proposal was the shortened stay at the host institution. Due to mandatory induction procedures for visiting researchers at Agriculture Victoria, nearly one week was lost before the applicant could access laboratory spaces. In addition, some covid-19 protocols were still enforced, which meant that most staff would only work every other day during the week. This sometimes became a problem when the applicant needed assistance or demonstrations of equipment etc.

Another unforeseen problem was the illness of key member of the host research group, which lost the project at least another week.

With regards to the experimental work at the host institution, it quickly became clear that the key assay – small hive beetle attraction to yeast within a wind tunnel set-up, was not yet reproducible. Therefore the initial plan to compare small hive beetle attraction to local vs. non-local strains of the yeast *K. ohmeri* had to be indefinitely postponed while efforts were made to standardize the wind tunnel attraction assay against a reference yeast (the regular baker's yeast *Saccharomyces cerevisiae*). However, there was no measurable success before the applicant had to leave the host institution.

However, one unexpected success of the project occurred prior to the trip to the host institution. During the first three weeks of the project, which spend at the home institution in Sweden, the applicant reviewed the genomic data used to design genomic markers that were meant for profiling yeast isolates to distinguish local isolates from non-local isolates. The initial proposal from 2019 had developed one specific genomic barcode based on the intron within yeast ribosomal protein gene *RPL18*. The *RPL18* intron sequence appears to mutate fast enough to resolve yeast isolates within a species. However, not all budding yeast species have introns in their *RPL18* genes, which meant that several key genera of insect-associated yeast (e.g. *Starmerella*) did not yet have any suitable candidates for intra-species barcoding.

During the review of yeast genomic data in August 2022, the applicant had access to a larger dataset than was available in 2019. As a consequence, a number of new candidate barcodes were characterized – some of which could be applied for genera with intron-less *RPL18* genes. A manuscript detailing this analysis and listing those genomic barcodes with most utility is currently being finalized and is likely to be submitted for publication during the summer of 2023.

3. Major achievements of the fellowship

As stated in the previous section, the main achievement of the fellowship was the identification and characterization of improved genomic barcodes for unambiguous species assignment as well as studies of intra-species diversity. While not directly relevant to the stated goals of the fellowship, these results will be of great use in all settings where rapid and precise identification of yeast species is of importance. This is particularly valuable for molecular diagnostics of pathogenic yeasts that infect humans (e.g. *Candida auris*) but may also be a good tool for the study of phytopathogenic yeasts (e.g. *Eremothecium*, *Taphrina*, *Ustilago*).

4. Follow-up work

The applicant still wishes to complete the stated research goals of the original proposal looking at olfactory responses in pest insects to yeast volatiles. Funding has already been secured for a return trip to the host institution, which is preliminarily scheduled for the end of 2023.

5. How might the results of the research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

While the ultimate practical objective of the proposed research project (i.e. improved lures for pest insects) is not yet achieved, the improved genomic barcodes identified as a result of the first phase of the amended project plan will be very valuable for molecular diagnostics of yeasts and perhaps other fungi as well. Typically, fungal population genetics require whole-genome sequencing and advanced skills in genome sequence assembly and analysis. By using short genomic barcodes, fungal diagnostic as well as studies of fungal intra-species variation

will become available to a greater spectrum of users that may lack the sequencing infrastructure and/or analytic expertise for genome-scale analyses. Yeasts and fungi affect all aspects of the agriculture and forestry sectors and as such, easy tools for species assignment are extremely important.

6. How was the research project relevant to the objectives of the CRP and the CRP research theme "Transformational technologies and innovation"?

The use of yeasts to attract pest insects though volatile compounds remains an intriguing approach in sustainable pest management. Had the stated goals of the project been achieved, it possible that much could have been learned about the mechanisms of olfactory attraction and the overall chemical ecology of yeasts and insects. Nevertheless, the fellowship succeeded in forging stronger connections between yeast science and pest insect science. It is clear that these two subject areas will need to continue collaborating across disciplines in order to address the current and future challenges of insect-mediated destruction of agricultural and forestry resources.

7. Satisfaction with the fellowship

Despite the many practical difficulties encountered during the execution of the proposed project, the applicant still feels that the experience was beneficial overall. Interactions with researchers at the host institution gave rise to many interesting project ideas to be pursued in future grant proposals. The applicant also found it very rewarding to assist in insect behavioural experiments, which are far away from the applicant's own area of expertise. In a sense, the applicant feels that the most important contribution of the OECD CRP program is to allow researcher separated not only geographically but also thematically to interact over an extended period of time.

8. Advertising the Co-operative Research Programme

The applicant learned of the OECD CRP through a colleague at the home university.