End of Studentship Report – Charlotte Drastich

Aims of the project

Determine which strain(s) of *Aspergillus oryzae* are best suited for recombinant food protein production using a Multiomics analysis approach.

Introduction

Microorganisms play a key role in attaining a more sustainable food production system. One such role is their ability to produce so-called 'microbial foods' which include fermented foods, microbial biomass, or products of microbial fermentations. I worked on a microbial foods project involving filamentous fungus *A. oryzae*, used in products like sake and soy sauce, involving products of microbial fermentations. My supervisor was a PhD student, Casper, whose project is focused on recombinantly producing rapeseed proteins using *A. oryzae* as a cell factory. As it is not fully established as a biotechnological tool, he is employing various analysis techniques to investigate *A. oryzae* as an expression system. During my time in the lab, I was involved in different areas of his project.

Studentship Summary

<u>Genomics</u>

42 strains from the NRRL Database were sequenced with BGI DNBseq. We adopted a workflow another researcher at the center had established to assemble and annotate the genomes of the 42 strains Casper previously sequenced, adding further annotations for our own purposes.

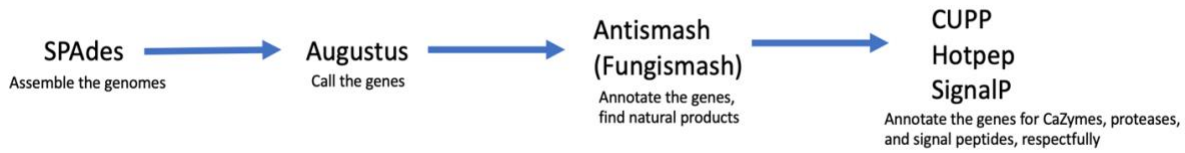


***Figure 1**: Annotation pipeline; the pipeline can be found on GitHub via pablo-genomes-to-vials-cruz.*

Table 1: *Gene annotation statistics after full pipeline showing number of genes annotated in the 42 NRRL Aspergillus oryzae strains.*

Strain	Number of Genes Called	Number of Genes Annotated	Percentage of Genes Annotated
NRRL_13765	11596	9113	78,6%
NRRL_1560	11535	9079	78,7%
NRRL_1958	11316	8926	78,9%
NRRL_1998	11526	9068	78,7%
NRRL_2217	11697	9189	78,6%
NRRL_2220	11680	9149	78,3%
NRRL_31119	11526	9043	78,5%
NRRL_32657	11691	9143	78,2%
NRRL_3484	11549	9097	78,8%
NRRL_3485	11611	9107	78,4%
NRRL_3486	11611	9111	78,5%
NRRL_3487	11593	9106	78,5%
NRRL_3488	11594	9112	78,6%
NRRL_3502	11593	9099	78,5%
NRRL_35191	11470	9030	78,7%
NRRL_35739	11572	9129	78,9%
NRRL_447	10579	7902	74,7%
NRRL_448	11502	9059	78,8%
NRRL_451	11502	9069	78,8%
NRRL_455	11550	9100	78,8%
NRRL_456	11527	9081	78,8%
NRRL_458	11507	9064	78,8%
NRRL_459	11677	9192	78,7%
NRRL_460	11544	9093	78,8%
NRRL_461	11318	9018	79,7%
NRRL_466	11541	9067	78,6%
NRRL_471	11499	9077	78,9%
NRRL_476	11423	9005	78,8%
NRRL_480	11460	9063	79,1%
NRRL_4803	11482	9016	78,5%
NRRL_481	11537	9039	78,3%
NRRL_484	11654	9151	78,5%
NRRL_506	11533	9120	79,1%
NRRL_552	10536	8016	76,1%
NRRL_5588	11607	9113	78,5%
NRRL_5589	11559	9085	78,6%
NRRL_5593	11580	9089	78,5%
NRRL_5938	11547	9093	78,7%
NRRL_6270	11552	9093	78,7%
NRRL_694	11544	9099	78,8%
NRRL_695	11555	9097	78,7%
NRRL_696	11550	9091	78,7%

Pan Genome Analysis will be run using the BPGA program. The results will be used to build a Phylogenetic Tree, illustrating the different clades of the Aspergillus species and the *A. oryzae* strains.

Currently, fungal genomes are spread across various databases with variable quality of sequence data. We created our own 'dataset' to make comparisons amongst strains so we can make informed choices on which strain(s) to investigate and use as a cell factory. Casper will continue this workflow by looking at nucleotide variances/population genetics, to see which genes are vital. The genomic information can then also be coupled with transcriptomics and secretomics to, providing information on strong/efficient secretion signals.

Phenotypic Microarrays

RIB40 strain spore suspensions were added to two Biolog PM1 plates; parafilm-covered and uncovered in case of overgrowth/sporulation. Area scans of 590nm and 790nm were measured every 6 hours for 96 hours. The negative control had growth- perhaps since spore suspensions were from PDA plates, media from the agar may have been transferred. For a second run, spores were washed in ddH2O, and PM1 plates with spore suspensions of FF and water ran concurrently. This was to test if the FF may cause growth. Both conditions had minimal growth in the negative control.

These microarrays will also allow us to validate and tweak our genetic models, because will show which 'potential gene products' are expressed and utilized. This in turn will aid in creating genome scale metabolic models.

Proteomics

Engineered strains producing rapeseed proteins and milk proteins were cultivated in CDG minimal media. Supernatant was run through Ni-NTA resin column to purify His-tagged proteins, and SDS-PAGE was run.

The aim was to run LC-MS to check recombinant proteins expression. However, no protein bands were visible in the SDS-PAGE. We could try to run a wildtype supernatant as a positive control to check our technique. Then, we can try re-engineering these strains. The parent strain may have mutations rendering them unsuccessful for transformations. MS will be important to check protein presence via its primary sequence because gels only show molecular weight.

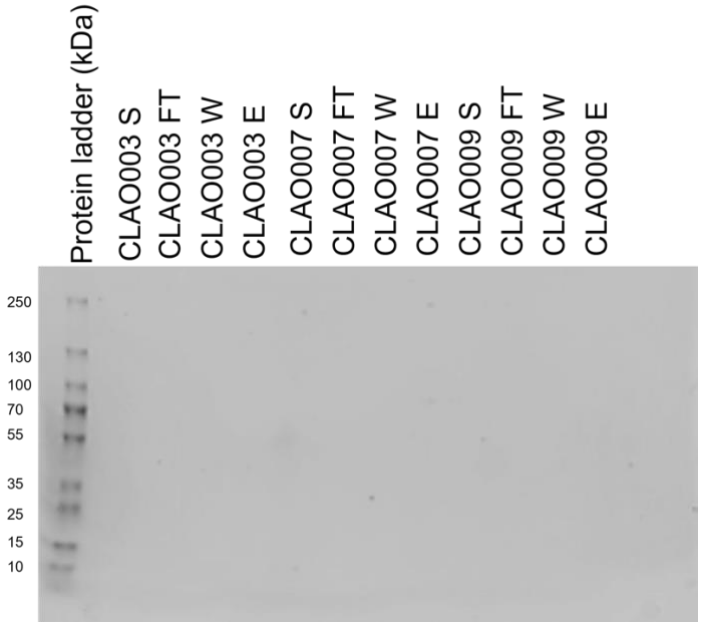


Fig. 2: *SDS-PAGE of the supernatant from the CDG Minimal Media. CLAOxxx refers to strains, S refers to supernatant, FT refers to flowthrough, W refers to wash, E refers to elution.*

Cloning and Transformations

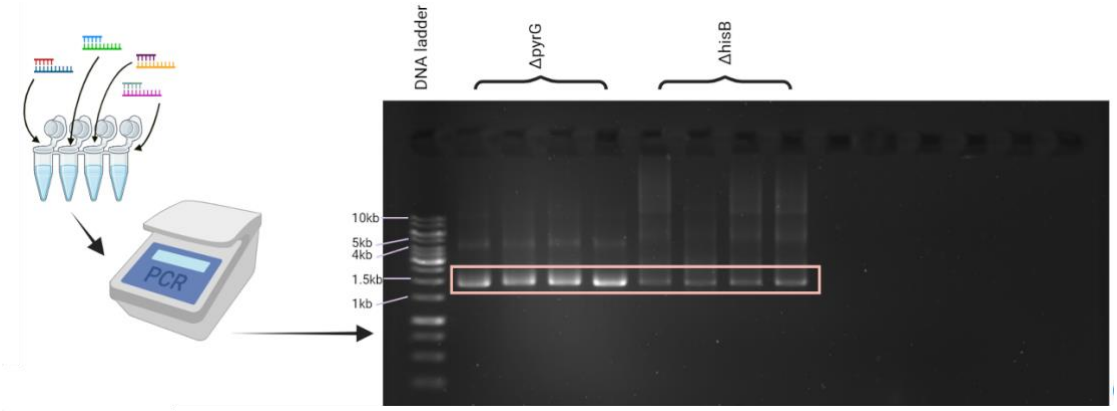


Fig. 3: *Cloning the linear fixing templates for the subsequent transformations, into reference strain RIB40. Agarose gel shows that PCR of the pyrG and hisB repair fragments was successful, shown by highlighted bands at ~1.5kb. Created with BioRender.com.*

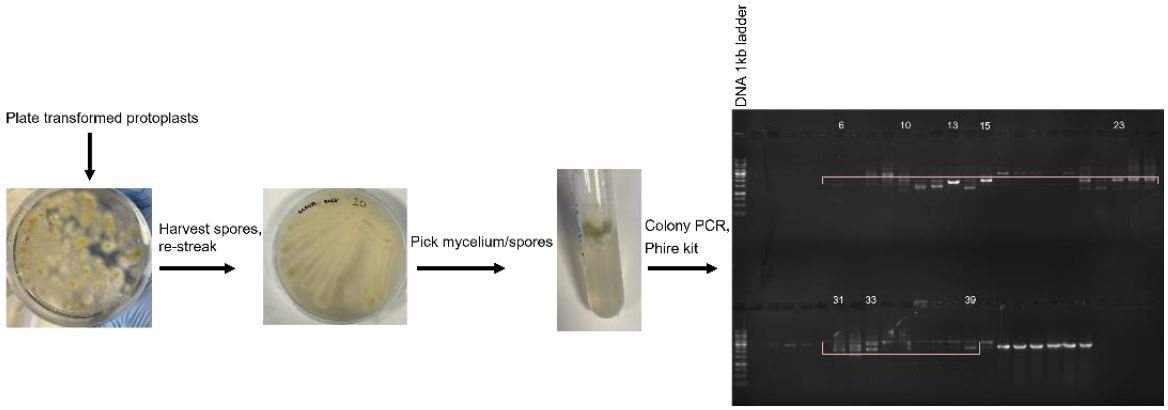


Fig. 4: *Workflow of transformation protocol, adapted from Leynaud-Kieffer et al, 2019. In agarose gel: lanes 1-25= ΔpyrG, 26-30=ΔhisB, 31-35= ΔpyrG, 36-40= ΔhisB, 41-46= ΔwA. Colony PCRs failed. Transformation protocol adopted from Leynaud-Kieffer et al, 2019.*

Perhaps we were too conservative when picking the biomass from the slants because marker selection seemed to work, otherwise there should not be growth on any plates. To test the counter selection, we can re-streak wildtype and mutant strains onto the respective plates and see if there is growth. Then, new round of transformations can be run to integrate GOI. The aim to have add another counter selection marker, to have a 3-marker strain for more efficient engineering.

Considerations

For the science community, this work was important because of *A. oryzae*'s untapped potential as a cell factory. It is currently utilized to produce enzymes, food, pharmaceuticals, secondary metabolites. Societally, we could benefit from this research with more sustainable food sources and other biological products to combat the current environmental crisis. This work falls under the synergistic, collaborative goals of the Biochemical Society, integrating different areas of science and different research groups to encourage links and networks for information exchange. Many private companies are conducting the same/similar research, and their results are not accessible due to patents; this work is promoting and sharing knowledge.

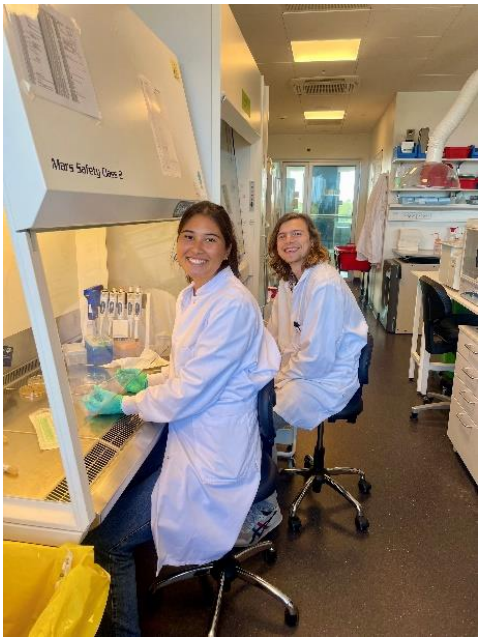
Value of studentship:

I learned how to work with filamentous fungus, an organism with growing widespread use in biotechnology. I solidified foundational technical skills including PCR, making plates and slants on large scales, primer/gRNA/repair fragment design, DNA/protein gels, using multichannel and automatic pipettes. Genomics was an opportunity to integrate dry with wet lab, which is representative of the interdisciplinary direction science is veering towards.

The research center is very collaborative. This allowed me to develop skills integral to collaboration: communication, organization, flexibility, teamwork, respect, being detailed-oriented.

Acknowledgements

Thank you so much to the Bacterial Synthetic Biology group for their help and support throughout the course of my project. Even though I was only there for a short time, I felt so welcomed and included. I would like to thank my supervisor, Casper, for sharing his knowledge and enthusiasm with me. I thoroughly enjoyed my experience, and it would not have been the same without him. I would also like to thank Dr. Leonie Johanna Jahn, because even though she was busy and on maternity leave she offered me this opportunity and helped me, alongside Tiffany and Casper, with my Studentship application. Thank you to Tiffany for helping with the application process and meeting with me to discuss initial steps. Thank you to the Biochemistry Society for giving me the opportunity to be able to travel and engage in such an amazing experience. I am so grateful to have been able to work abroad and learn about research that is more applicable/industry-oriented but still in an academic setting. I am so fortunate to have been part of The Microbial Foods Lab this summer. Thank you to everyone who was part of this experience :)



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