Exploring pathogenic fungal communities within the rhizosphere of *Brassicas*

This summer I was involved in a project at the University of Glasgow that is assessing the rhizosphere microbial communities associated with three species of *Brassica* that have varying levels of ploidy: the allotetraploid *B. napus* and its diploid progenitors *B. oleracea* and *B. rapa*. It is thought that individual plants may be able to select these communities via root secretions. Furthermore, it is likely that the composition of these secretions vary between species and ploidy-level, thus selecting for and against different types of microorganisms in order to create the most beneficial rhizosphere microbiome for the plant. As these microorganisms will predominantly be selected from the soil in which plants grow, my project focused on the differences in root-associated (rhizosphere) fungal communities between plants grown in two different common garden environments as well as between species.

DNA from rhizosphere soil samples was extracted, and then amplified using the fungal primer set LSU200A-F/LSU476A-R. This is a recently proposed set of fungal primers that target the D1 variable region of the nuclear large subunit (LSU) of the ribosomal DNA array. The amplicons were then cloned and transformed into bacterial cells. Those bacterial cells that were identified as having taken up the fungal DNA had their DNA extracted and were sent for Sanger sequencing. Sequence data was cleaned up using the programme Sequencher and the fungal species were identified using BLAST to query GenBank. This gave us an idea of what fungi are present in the rhizosphere of the different species in the two environments.

Based on characterisation of phyla it was evident that each individual possessed different fungal communities, including differences in prevalence of some known pathogens. Additionally, although these primers were designed to target the phylum Ascomycota and be used in combination with another set (LSU200-F/LSU481-R) targeting all other fungi, I found that LSU200A-F/LSU476A-R are also capable of amplifying fungi from Zygomycota, Chytridiomycota, and Basidiomycota. Although they also amplify algae, amplification of host plant DNA was rare. Overall these primers proved effective in fungal pathogen identification that could be used in high throughput approaches using next generation sequencing; further investigations into the pathogens found in the different species could provide an insight into the specific component(s) of root secretions that diminish pathogen survival rates the most. This knowledge may have useful applications in crop defense against pathogen infections.

I would like to thank the BSPP for funding my project. This has allowed me to be involved in a professional lab environment where I have learnt many new skills and techniques. Furthermore, I have gained invaluable experiences that have given me the confidence and a greater interest in pursuing a career in plant pathology. I would also like to thank my supervisor Professor Barbara Mable, her PhD student Elizabeth Mittell, and a special thanks to Elizabeth Kilbride, who have all taken the time to teach me essential skills in working in a lab as well as several important techniques in data analysis.

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