

## Rhizosphere profiling using deep sequencing

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Locations: Ramsay lab CHIRI and TrEnd lab in School of Molecular Sciences, Curtin. Some work at Centre for Rhizobium studies (CRS) Murdoch

Legume crops such as Chickpea are grown throughout Australia but as they are not native to Australia most require inoculation with specific bacterial symbionts. These bacteria called mesorhizobia form a nitrogen-fixing symbiosis within plant roots structures called nodules, where they supply nitrogen to the plant. The efficacy of the inoculant strain depends on multiple factors. These include nodule-formation and nitrogen-fixation rates and the strains competitiveness, persistence and survival in the soil. The genes for nitrogen fixation and symbiosis are found on large (up to 600 kilobases) DNA regions called integrative and conjugative elements (ICEs). These regions can excise from the mesorhizobial chromosome and horizontally transfer to non-symbiotic mesorhizobia in the soil, converting native soil mesorhizobia into new legume symbionts. These newly-evolved strains vary widely in their performance and in some cases form ineffective nodules that outcompete inoculant strains and decrease agricultural productivity. Until recently, nothing was known about where non-symbiotic mesorhizobia came from. Using selective culture techniques we have discovered non-symbiotic mesorhizobia are abundant and diverse in Australian soils and can even be readily isolated from dirt at Curtin University! We have isolated and draft-sequenced the genomes of ~100 non-symbiotic mesorhizobia. We will now develop high-throughput techniques to explore the diversity of mesorhizobia in Australian soils more deeply. This will enable comparison of relative abundance of mesorhizobia in soils, relative clade abundance at different sites, abundance of symbiosis ICEs and ICE-carrying strains before and after inoculation, and the relative abundance of strains in nodules. This project involves developing standardised workflows for DNA extraction and highthroughput amplicon sequencing using Illumina short-read sequencing technologies. This work forms part of a multi-million dollar GRDC and ARC-funded collaboration between the Ramsay lab and the Centre for Rhizobium studies (Murdoch).