Investigating the transcriptomic response of *E. adhaerens* during the colonization of *A. thaliana* root tissues

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Ensifer adhaerens is a gram-negative soil borne bacteria with an addressed capability to transfer genetic material into monocot and dicot cells (Zuniga et al, 2015; Wendt et al, 2012).The interaction of a bacterium with its host plant is a complex process which involves potential synergistic or antagonistic mechanisms. However, these mechanisms still remain unknown for *E. adhaerens*. In reply, the goal of this study is to obtain a comprehensive analysis of the transcriptome of *E. adhaerens* in response to colonization/interaction with *A. thaliana* root tissue.

Firstly, preliminary transformation experiments evaluated the level of colonization of *E. adhaerens* on *A. thaliana* roots by histochemical GUS staining. This identified an optimal frequency of transient transfer at 5 dpi. Capitalising on this information for the present study, 20 day old roots of *A. thaliana* were cut into 2-3 mm lengths and inoculated with *E. adhaerens* (or media control), containing the plasmid pCAMBIA 5105. At set time points (0h, 24h 48h, 72h, 5 and 7 days) total RNA was harvested from collected bacteria cells, with three biological replicates completed per treatment and the experiment conducted twice. RNA seq is currently being completed by an external provider on an Illumina HiSeq 4000 system and data analysis is underway. Whole prokaryotic transcriptome profiles distinct to this plant-bacterium interaction will be presented.

Separately; using the qRT-PCR and the deltaCt method (Livak and Schmittgen, 2001), transcriptional profiles of four selected target genes; *pcs* (phosphatidyl choline synthase), *trbI* (conjugal transfer protein), *katA* (catalase A) and *chvD* (chromosomal virulence D) were investigated due to their potential role in the ability of *E. adhaerens* to transfer T-DNA to plant cells (Rudder et al, 2015). The expression levels of the three genes *pcs*, *trb* and *kat-A* peaked at day 5 showing fold changes of 11.0, 8.2 and 6.7 respectively before decreasing at day 7, with relative fold changes of 3.5, 3.1 and 3.3 respectively. In contrast *chvD* expression peaked at day 7 with a relative fold change of 114.5. The correlation of the overexpression of the *pcs*, *trb* and *kat-A* genes at day 5 with the GUS phenotypic data during the same time course would indicate an associative role for these genes in regard to T-DNA transfer by *E. adhaerens* into plant cells. The compilation and analysis of the RNAseq data will provide additional insight on the functioning of the *E. adhaerens* genome during the process of cellto-cell T-DNA transfer.

1. Zuniga-Soto E, Mullins E, Dedicova B. Ensifer-mediated transformation: an efficient non-Agrobacterium protocol for the genetic modification of rice. *Spriger plus*. 4(600)
2. Wendt T, Doohan F, Mullins E (2012) Production of Phytophtora infestance resistance potato (Solanum tuberosum) utilizing Ensifer adhearence OV 14. *Trans Res* 21:567–578
3. Schmittgen T and Livak K. Analyzing real-time PCR data by the comparative CT method. *Nature Protocols* 3, -1101 – 1108
4. Rudder S, Doohan F, Creevey CJ, Wendt T, Mullins E (2014) Genome sequence of Ensifer adhaerens OV14 provides insights into its ability as a novel vector for the genetic transformation of plant genomes. *BMC Genom* 15:268. doi:10.1186/1471-2164-15-268