Investigating lifestyle changes in *Rhizobium leguminosarum*

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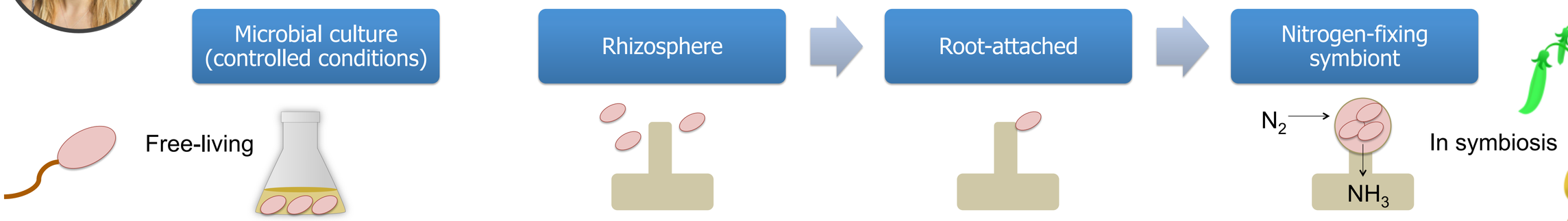

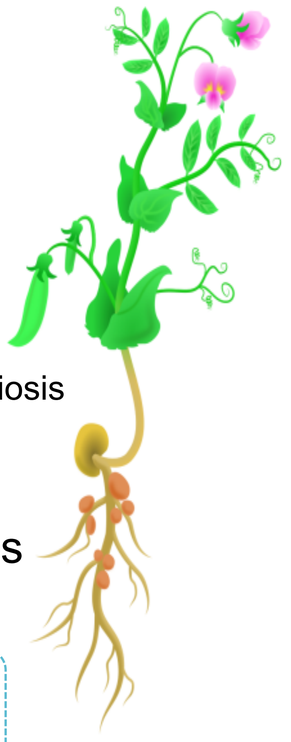

High-throughput Insertion Sequencing (INSeq) genomics has been used to investigate different lifestyles in *Rhizobium leguminosarum* bv. *viciae* 3841 (Rlv3841) on the whole-genome scale

Fitness values for 7,316 genes (99.7% of genome) quantified in INSeq screens [1,2]
Accuracy of gene fitness predictions validated by directed mutational studies
Reliable genome screening optimised in microbial cultures before moving onto more complex plant-influenced environments

1. The role of O_2 in growth on glucose and succinate

INSeq analysis of Rlv3841 grown on glucose or succinate at both 21% O_2 and 1% O_2 was used to understand how O_2 concentration alters metabolism [3].

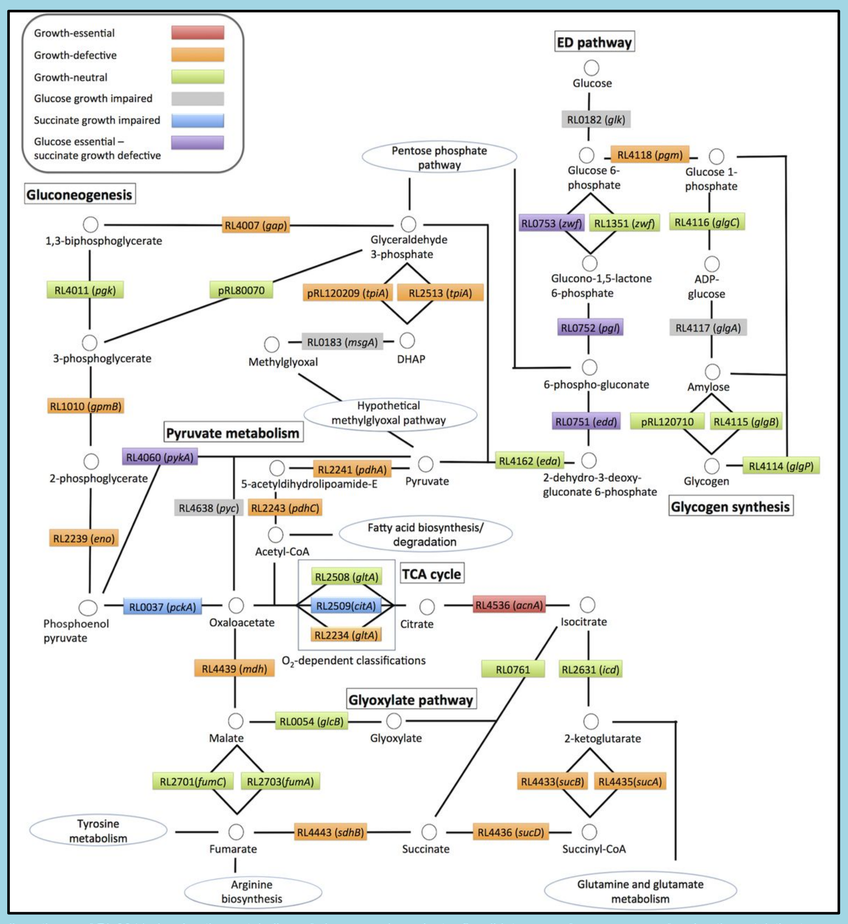

Figure 1. Central metabolic pathway of Rlv3841 showing the metabolism of glucose and succinate. Candidate genes for enzymes performing the catalytic steps are shown in colored boxes according to their INSeq mutant classification: red, growth essential (ES) under all growth conditions; orange, growth defective (GD) under all growth conditions; green, growth neutral (NE) under all growth conditions; gray, growth impaired on glucose, i.e., growth essential or growth defective specifically on glucose; blue, growth impaired on succinate, i.e., growth essential or growth defective specifically on succinate; purple, growth essential (ES) on glucose and growth defective on succinate.

The ED pathway was shown to be essential for growth on glucose, as expected, but surprisingly, its mutation caused growth deficiency on succinate → suggesting that sugars made by gluconeogenesis must undergo recycling.

TCA cycle essential for growth on both glucose and succinate. Most genes encoding TCA cycle enzymes showed the same INSeq mutant classification on both carbon sources.

Rlv3841 appears to use the methylglyoxal pathway alongside the ED pathway and TCA cycle for optimal growth on glucose.

A novel toxin-antitoxin system was identified on pRL10 that could be important for generation of new plasmidless rhizobial strains.

2. The influence of nitrogen and carbon source on O_2 consumption and growth rate

The mean generation time of Rlv3841 on succinate at 1% O_2 was almost double that of the other three growth conditions (Figure 2). Growth on succinate was also seen to require around double the oxygen consumption rate compared to glucose (Figure 3).

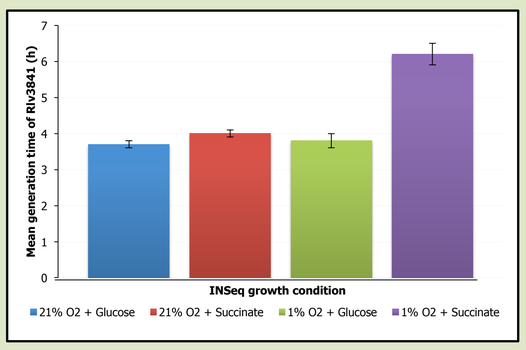

Figure 2. Mean generation time of Rlv3841 calculated as the number of hours it took the population to double while in exponential growth phase (5 biological replicates).

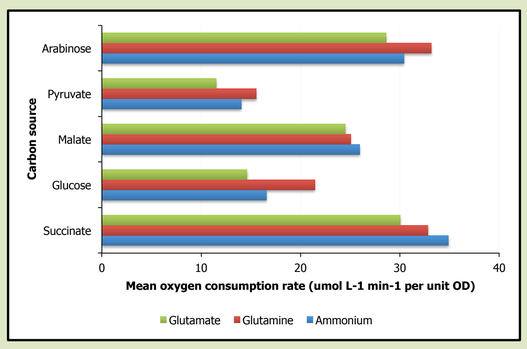

Figure 3. Mean oxygen consumption rate for Rlv3841 growth on varied carbon and nitrogen sources provided in minimal media (9 biological replicates).

There is a significantly higher oxygen consumption rate for cells growing on direct TCA cycle intermediates (succinate and malate) than on non-TCA cycle intermediate carbon sources (glucose and pyruvate).

Across all carbon sources it was universally observed that cells growing on ammonium have a higher oxygen consumption rate than those growing on glutamate.

Growth substrate → TCA cycle activity → reductant reoxidisation requirements → oxygen consumption rate

3. Fitness determinants for the colonization of pea roots in nitrogen-fixing symbioses

INSeq analysis of Rlv3841 grown in the rhizosphere of its host legume pea (*Pisum sativum*) and attached to roots was used in the novel genome-level characterisation of the fitness determinants of attachment and colonisation,

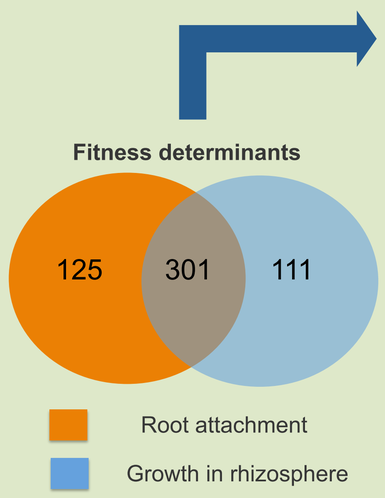

Figure 4. Number of gene mutants classified as growth-essential or growth-defective specifically in the root attached or growth in the rhizosphere INSeq libraries

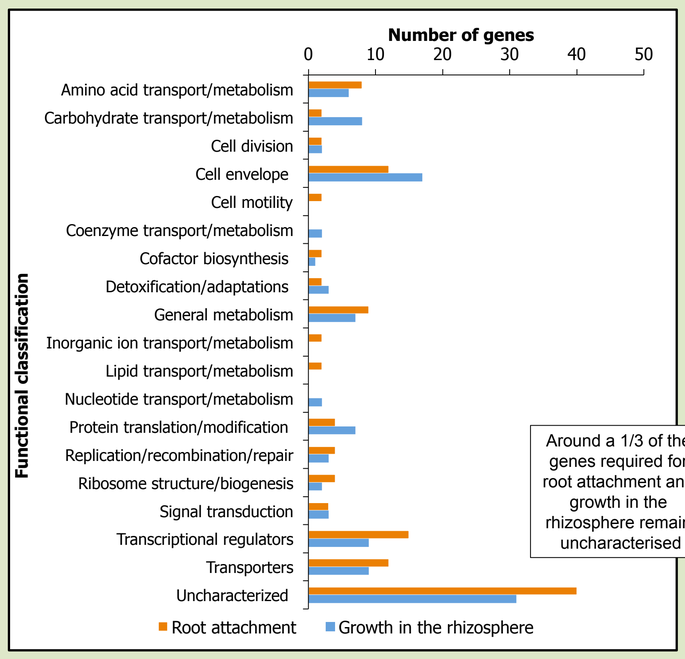

Figure 5. Functional classifications of the genes required specifically for root attachment and growth in the rhizosphere (Figure 4).

Representation of fitness determinants across the Rlv3841 genome

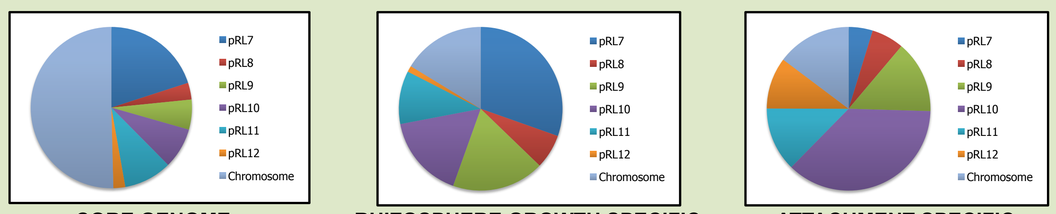

Figure 6. Proportion of genes in the core genome (total 545 genes) vs. rhizosphere specific group (total 111 genes) vs. attachment specific group (total 125 genes) in each replicon. This was standardised for replicon size as a calculation of the number of genes required relative to the total number of protein-encoding genes on the replicon.

As expected, the highest proportion of the core genome is found on the chromosome.

Plasmids play a more significant role in rhizosphere growth and attachment.

pRL10 (the 'symbiosis' replicon) plays the most significant plasmid role in root attachment.

A surprisingly high proportion of growth-defective/essential gene mutants are found on pRL7.

4. Mapping the transcriptional regulatory network in root attachment

Fourteen transcriptional regulators were identified as being specifically required for root attachment (Figure 5). The downstream targets of 13 of these regulators are uncharacterized.

Made Rlv3841 mutants of the 14 transcriptional regulator genes

↓

RNAseq carried out to obtain transcriptomic profile of mutants vs. wild-type

↓

Characterised the regulon of each regulator gene

↓

Lux-based reporter systems used for target validation + spatial and temporal activation analysis [4]

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Putative mapping of the transcriptional regulatory pathways in root attachment

Figure 7. Experimental work done to map the transcriptional regulatory network in root attachment

The pRL80032 LysR family transcriptional regulator was identified as a particularly important candidate in root attachment and further characterised. A mutant strain of pRL80032 demonstrated a striking phenotype indicative of significant alterations to the cell surface. Decreased fitness of the mutant in competitive root attachment was validated in mass competition experiments where attachment to roots was quantified with DNA sequencing (the mutant underwent a 1000-fold decline in proportion in the population from inoculation to retrieval from the roots after 5 days.

References

[1] Perry, B. J., & Yost, C. K. (2014). *BMC microbiology*, *14*(1), 1.
[2] DeJesus, M. A., & Ioerger, T. R. (2013). *BMC bioinformatics*, *14*(1), 303.
[3] Wheatley, R. M., et al. (2016). *Journal of bacteriology*, 199(1), e00572-16.
[4] Frederix, M., et al. (2014). *Molecular microbiology*, *93*(3), 464-478.