

Comparison of *Rhizobium leguminosarum* determinate and indeterminate nodules on legumes by RNA-Seq analysis

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Background

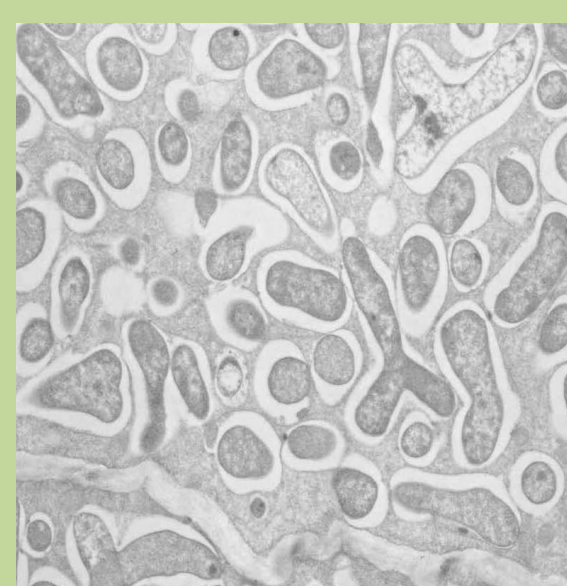
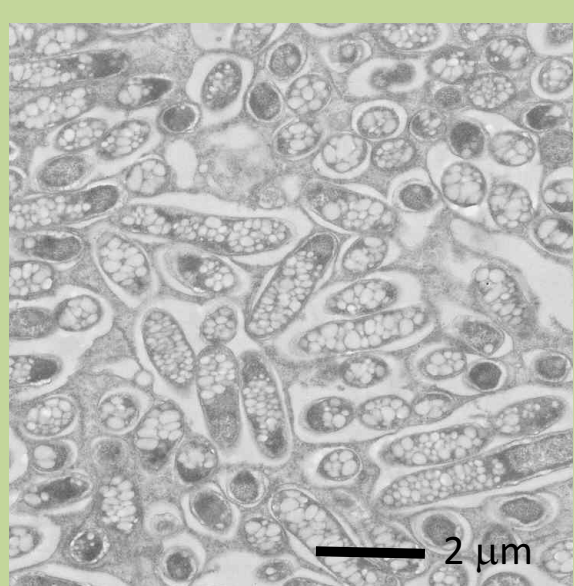
Strains of *Rhizobium leguminosarum* biovar *phaseoli* form nitrogen-fixing nodules on *Phaseolus vulgaris* (common bean). The ability to nodulate a particular plant is specified by the symbiosis (Sym) plasmid. Previously the Sym plasmid (pRP2) of *R. leguminosarum* bv *phaseoli* strain 4292 (Rlp4292) was replaced by the pea/vetch nodulation-specific Sym plasmid, pRL1J1, to make *R. leguminosarum* bv *viciae* strain A34 (RlvA34) (1).

With the exception of the Sym plasmid, these two strains have an identical genome composed of a chromosome and three plasmids. Their Sym plasmids confer the ability to nodulate either bean or pea plants, which form intrinsically different nodule types.



In bean, determinate nodules are formed which do not maintain an active meristem and undifferentiated bacteria can be cultured from mature nodules.

Galeoid legumes, such as pea (*Pisum sativum*), form indeterminate nodules where the bacteria are terminally differentiated, showing altered cell shape and a highly endoreduplicated genome (2).

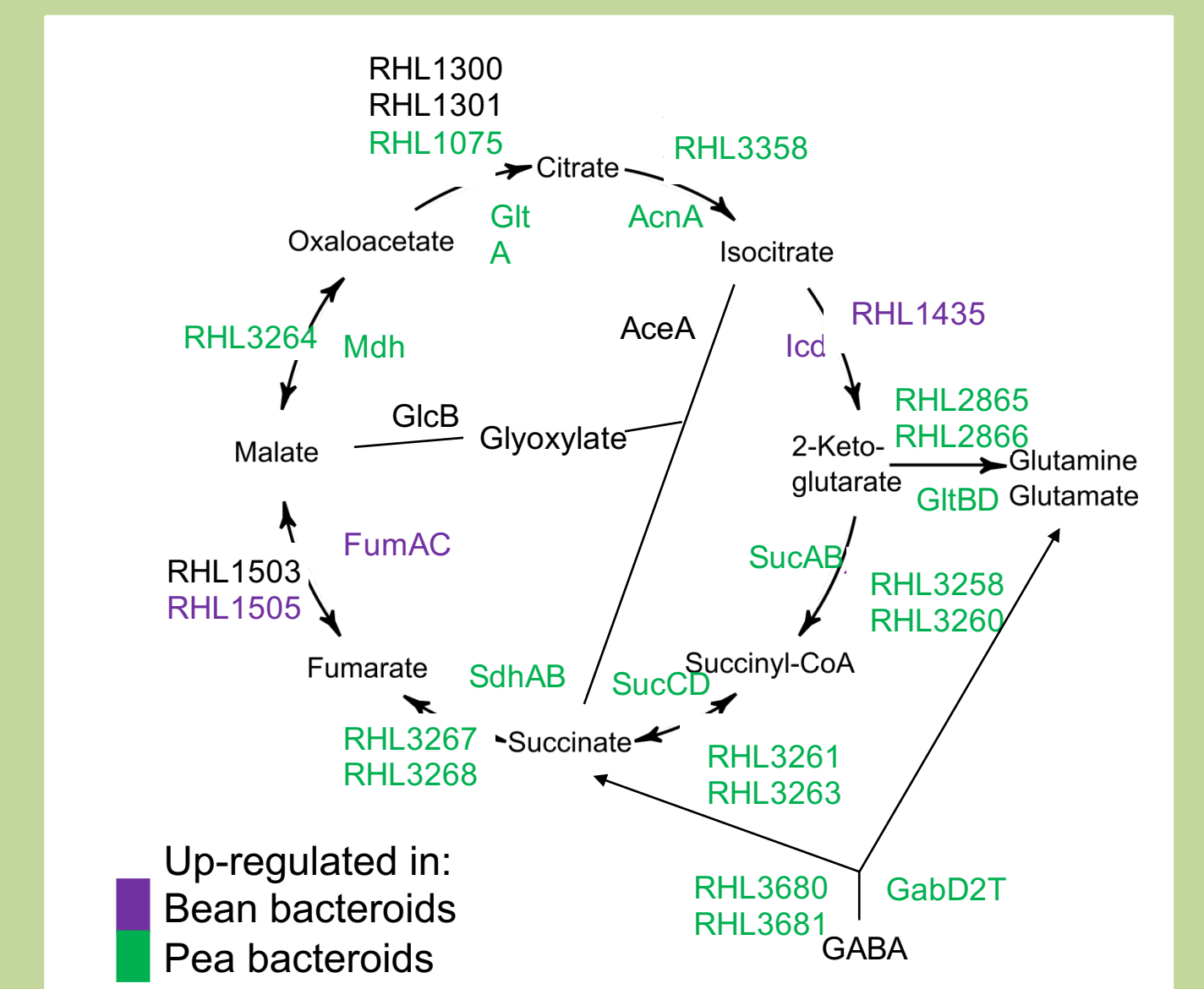


Changes to central metabolism in bacteroids

In bean bacteroids, expression of genes encoding the TCA cycle enzymes and those of associated metabolic reactions are up-regulated approx. 2-fold compared to free-living bacteria.

In pea bacteroids there is an approx. 10-fold up-regulation of expression of these genes (shown in green).

As free-living bacteria were grown on succinate, the TCA cycle is clearly very active in both bean and pea bacteroids, although induction of gene expression is higher in those of peas.



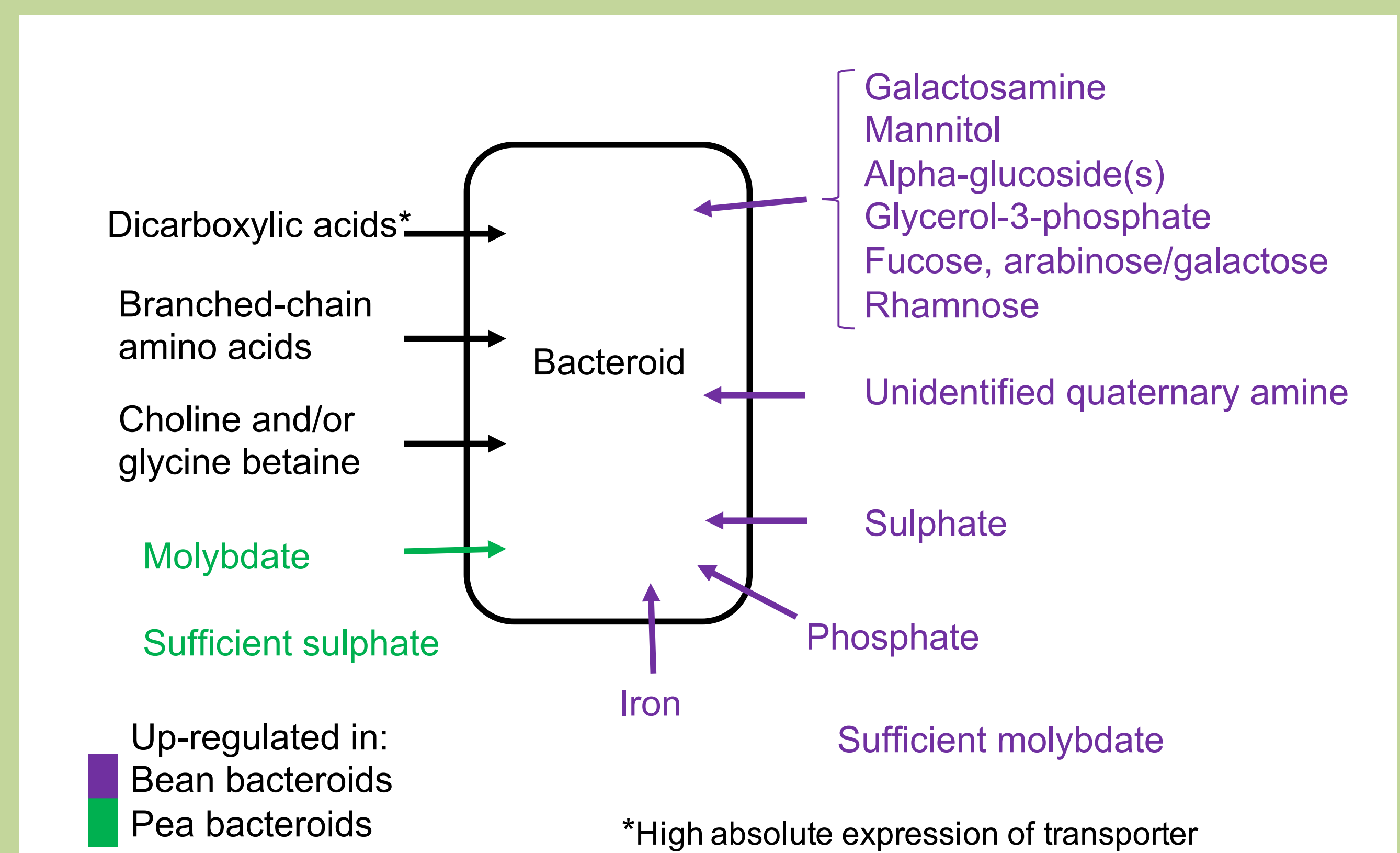
Determining bean and pea nodule environments through expression of transporter systems

In bacteria, genes encoding many solute transport systems are up-regulated in the presence of the solute – e.g. sugars, amino acids, organic acids.

In contrast, some transporters are induced in response to a perceived limitation, in order to scavenge for ions that the cell is lacking – e.g. phosphate, molybdate, iron.

This approach was used to identify the solutes transported by *Sinorhizobium meliloti* ATP-binding cassette (ABC) and tripartite ATP-independent periplasmic (TRAP) transport systems (3).

We examined the transporter genes up-regulated in Rlp4292 bean and RIA34 pea bacteroids and gained information on their respective nodule environments.



RNA-Seq analysis

Rlp4292
free-living & mature bean nodules

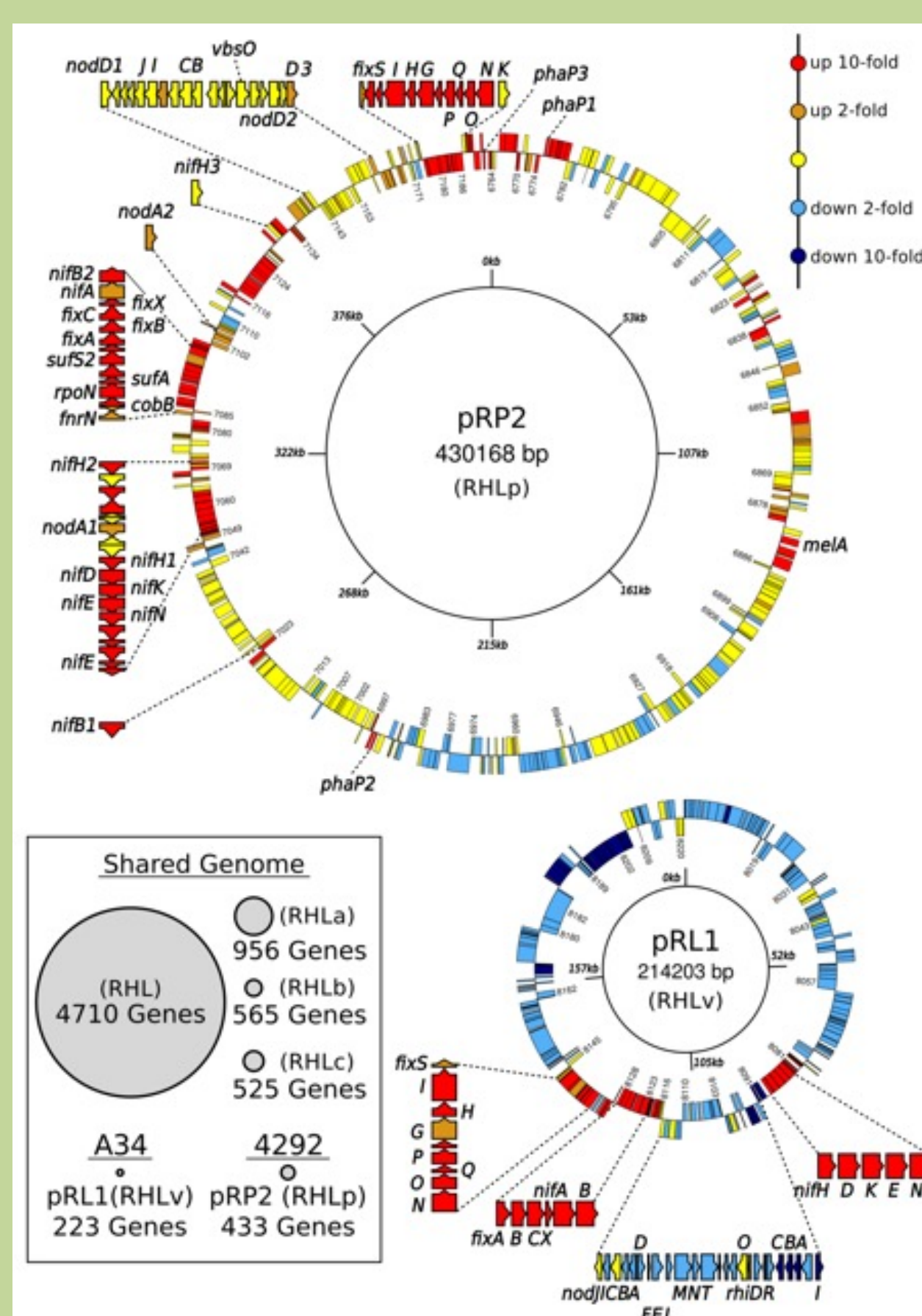
RlvA34
free-living & mature pea nodules

Genes differentially expressed in Rlp4292 and RlvA34 bacteroids compared to free-living:

COMMON to both determinate and indeterminate mature nodules

DIFFERENT in determinate and indeterminate mature nodules.

Differential expression of Sym plasmid genes during symbiosis



During symbiosis, many of the most strongly up-regulated genes are on Sym plasmids.

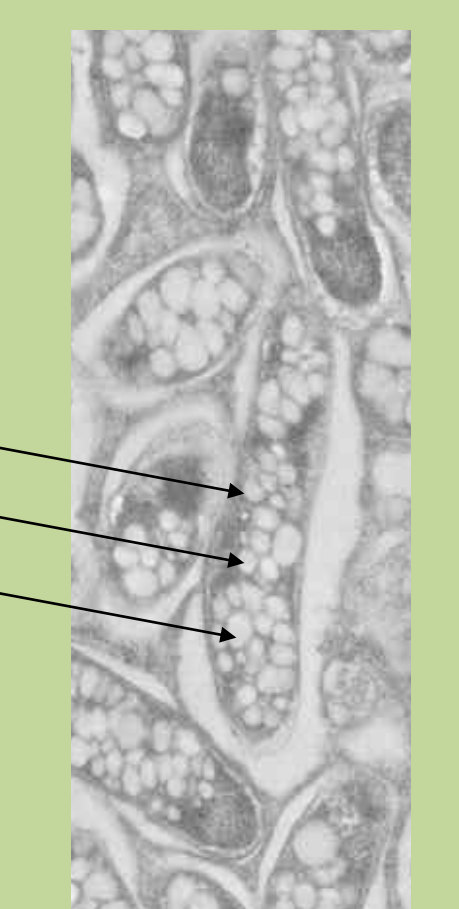
In bean and pea bacteroids, nitrogen fixation genes of *nif* and *fix* clusters are highly up-regulated.

The *nod* genes are not induced in mature nodules, suggesting that there is little contamination from either the rhizosphere or infection threads, (conditions where they are up-regulated strongly).

Large portions of pRL1 are down-regulated. This Sym plasmid has been added to make a synthetic strain, which is none-the-less able to nodulate peas

Other key findings

- In bean bacteroids there is specific up-regulation of the phasin genes (encoded on Sym plasmid pRP2) involved in coating granules of the storage polymer polyhydroxybutyrate (PHB). PHB can be seen (white granules) within mature determinate bean nodules.
- In pea bacteroids only, the expression of genes encoding numerous export systems is elevated. This could be due to exposure to plant-produced nodule-specific cysteine-rich (NCR) peptides which only indeterminate nodule bacteroids are exposed to.



Overall

Comparison of bacteroid gene expression in these two strains, identical except for the Sym plasmids, has enabled the differences and similarities between determinate (bean) and indeterminate (pea) nodules to be thoroughly investigated.

References

- (1) Lamb *et al.*, 1982 Mol. Gen. Genet. **186**:449-452.
- (2) Mergaert *et al.*, 2006 Proc. Nat. Acad. Sci. **103**:5230-5235.
- (3) Mauchline *et al.*, 2006 Proc. Nat. Acad. Sci. **103**:17933-17938