Understanding the role of FixABCX in nitrogen fixation in the *Rhizobium leguminosarum* symbiosis

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**Background**

- *R. leguminosarum* bv. *viciae* 3841 induces nodules on peas (*Pisum sativum*). Inside these nodules the bacteria differentiate into ‘bacteroids’ and carry out nitrogen fixation. Bacteroids require low oxygen concentrations for nitrogenase to catalyse nitrogen fixation. Nitrogenase catalyses the following reaction: 
  \[ N_2 + 8e^- + 8H^+ + 16ATP \rightarrow 2NH_3 + 4H^+ + 16ADP + 16P_i \]
- The rhizobial *fixABCX* genes are essential for nitrogen fixation in rhizobia. Their role is not characterised, although their products have homology with mammalian electron transfer proteins¹ and electron transfer proteins found in free-living nitrogen fixing species².
- *fixABCX* exist as an operon within *R. leguminosarum*. The genes are thought to be regulated by NifA, a general transcriptional activator of nitrogen fixation.
- My aim is to understand the role of the FixAB proteins within rhizobial symbioses and understand their transcriptional regulation.

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**A model for the role of FixABCX**

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**Mutating the *fixABCX* operon abolishes nitrogen fixation**

In frame *fixAB* mutants strain have been made to ensure the *fixCX* genes are expressed. Additionally, a *fixAB* deletion mutant strain was made, using a spectinomycin² cassette insertion, which effectively knocks out the entire *fixABCX* operon.

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**Using the lux system as a gene expression indicator**

The *fixABCX* promoter region coupled to LuBCDARE, allows screening for Fix gene expression under varying conditions.

Luminescence can be seen in nodules at 4 weeks post inoculation. Luminescence is not seen in free-living cultures.

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**The *fixABCX* genes are found as a single transcriptional unit in *R. leguminosarum***

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**fixABCX mutants show different bacteroid morphology**

Nodule phenotype and light and electron micrographs of *fix* mutants at 28 days post-inoculation.

*fixAB-Δspec* bacteroids are swollen and non-uniform. *ΔfixAB* bacteroids are extremely small although uniform. *ΔfixAB* bacteroids form small, electron-dense granules whose nature is being investigated.

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**Low oxygen does not activate *fixABCX* gene expression**

Chemostat analysis was used to investigate induction of the *fixABCX* genes, grown under limiting oxygen. qRT-PCR revealed that while *fixN*, a known oxygen responsive gene, is up-regulated *fixA* is not. Thus low oxygen is not the sole activator of *fix* gene expression.

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**Future Work**

- Step and FLAG tagged FixAB proteins are purified from bacteroids to study their electron transfer characteristics and interacting partners.
- The electron dense granules within *fixAB* bacteroids, suspected to be either lipids or polyphosphate granules, are being investigated.
- Additional signals that regulate *fixAB* expression are being investigated under microaerobic growth.

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References: