Understanding the role of FixABCX in symbiotic nitrogen fixation

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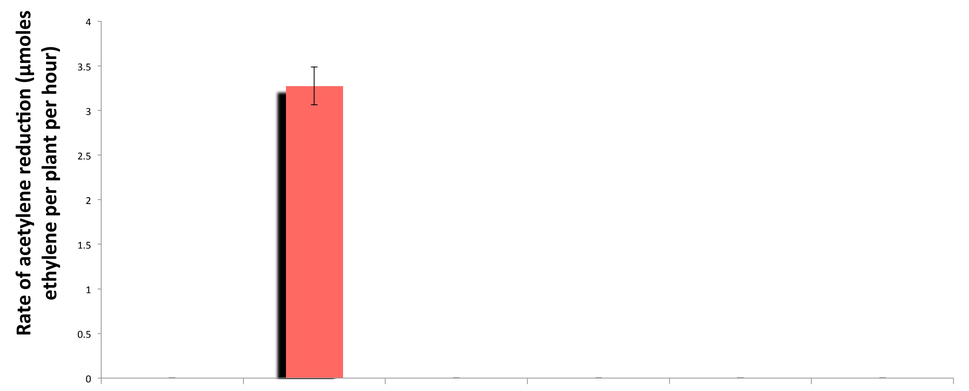
R. leguminosarum bv. viciae 3841 induces nodules on peas (*Pisum sativum).* Inside these nodules the bacteria differentiate into specialised bacteroids, fixing nitrogen in return for plant sugars. The fixation reaction only occurs under low nitrogen and low oxygen levels and is extremely energy demanding.

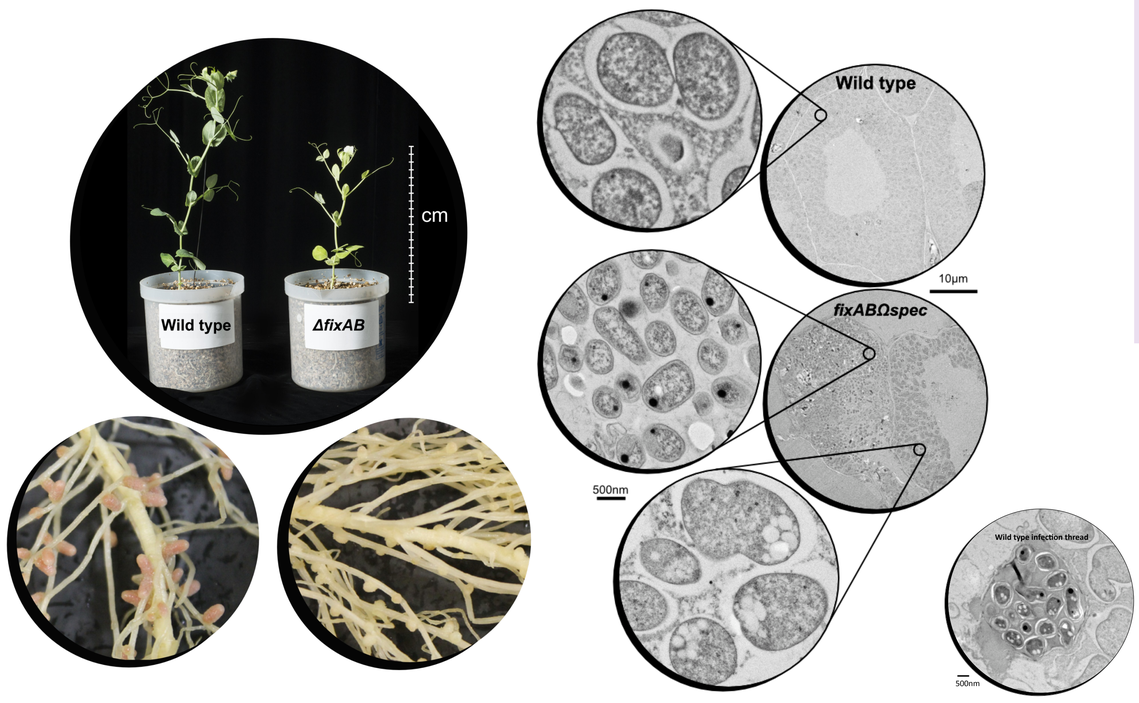
$$N_2 + 8e^- + 8H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

Within the differentiated bacteroids several genes are upregulated to express the machinery required for nitrogen fixation This includes an operon of four genes encoding the FixABCX proteins. The role of FixABCX has not been previously characterised within this symbiosis, although their products have homology with mammalian electron transfer proteins[1] and electron transfer proteins found in free-living nitrogen fixing species[2].

Mutants in *fixABCX* have abolished nitrogen fixation, confirming their essential role within symbiosis.

Acetylene reduction assay for nitrogenase activity

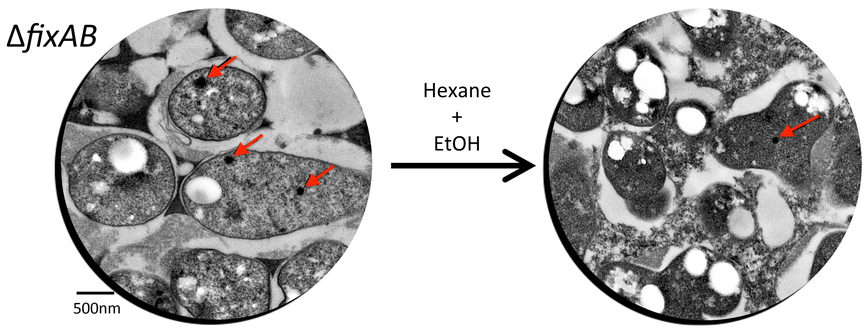




fixAB mutants show different bacteroid morphologies to the wild type

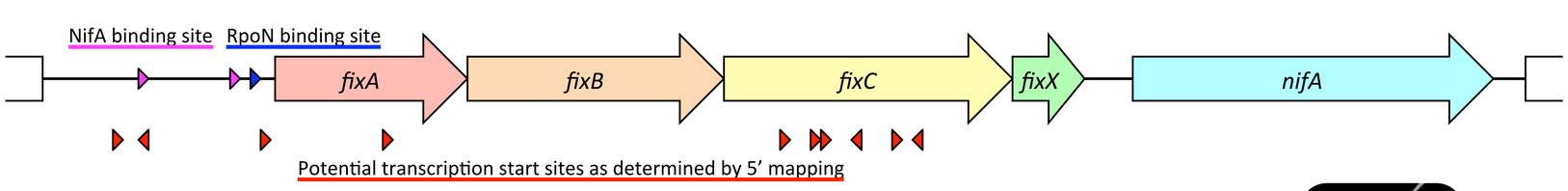
Bacteroids with a *fixAB* deletion desmonstrate clear morphological differences from the wild type, as well as between cells. Some plant cells contain smaller bacteroids with a high number of small, polar granules thought to be polyphosphate. Other plant cells contain bacteroids which can be dramatically larger than those of the wild type These larger cells appear to overproduce polyhydroxybutyrate (PHB), a storage molecule. In the wild type both polyphosphate and PHB appear most commonly in the developing infection thread.

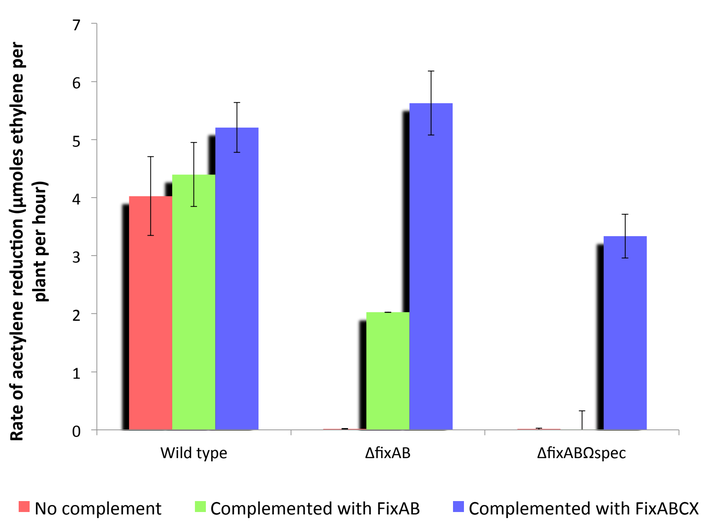
Transmission electron microscopy can be used to investigate the morphology of bacteroids. Treatment with hexane and ethanol is used to remove lipids



The *fix* operon is thought to be under the control of NifA, a general transcriptional regulator of nitrogen fixation.
NifA activity is environmentally controlled, active only in the bacteroids, where it activates expression of genes including the *fix* operon *and* those encoding the nitrogenase enzyme.

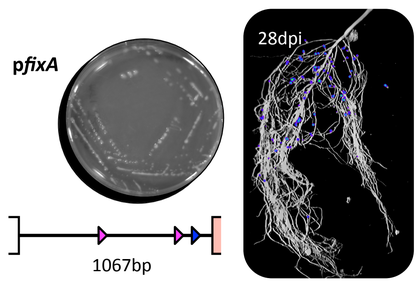
In some rhizobial strains *nifA* is autoregulated from a site upstream of *fixA*[3]. Complementation of *fix* mutants demonstrates that this is not the case in 3841.



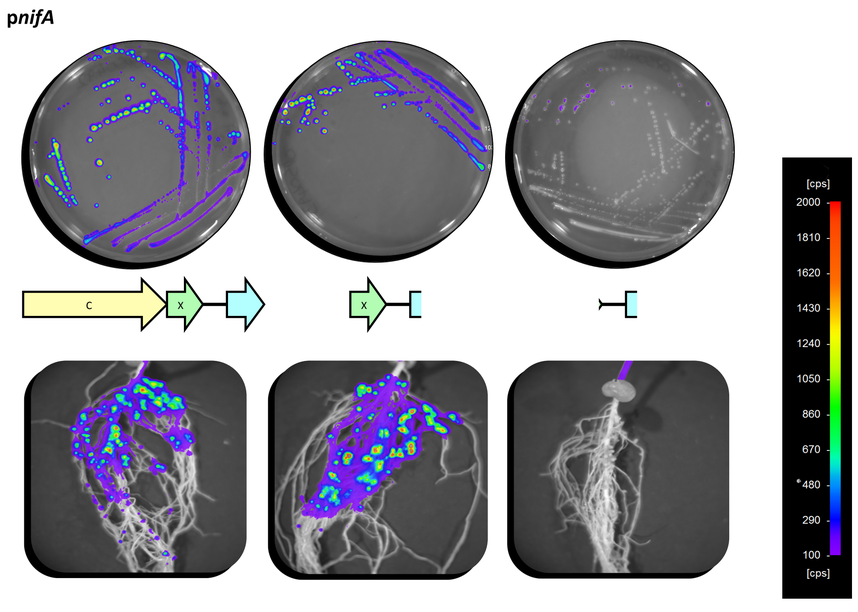


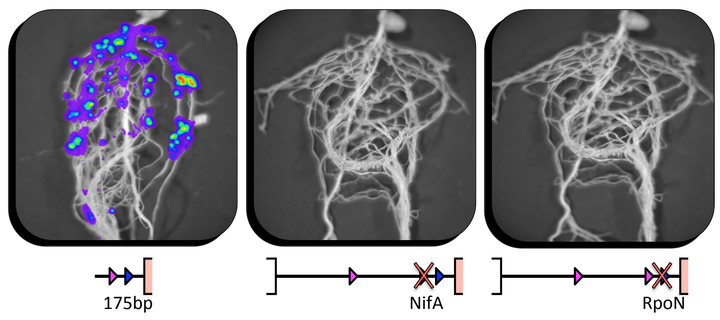
Using a bioreporter to investigate gene expression

A bioreporter system has been developed using the lux gene products. In this vector the promoter of interest is cloned ahead of the *luxCDABE* genes. If the promoter is switched on, luciferase is produced. This allows for non-invasive screening of promoter activity under different conditions.



nifA promoter activity originates inside the _fixX_ gene



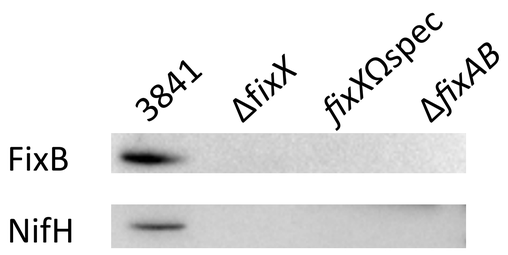


Expression of the *fix* operon requires NifA and sigma factor binding

Truncation and deletion of p*fix* demonstrates the essential features of the promoter region

The other NifA site may serve the divergent gene pRL100201, which is upregulated in bacteroids but has no characterized function.

fixX and _fixAB_ mutants do not express FixB or NifH in bacteroids



Conclusions

- The *fix* operon is essential for symbiotic nitrogen fixation between *R. leguminosarum* 3841 and *P. sativa.* Mutations in the *fix* operon show differing phenotypes as bacteroids develop, demonstrating production of storage metabolites.
- The lux bioreporter system can be a valuable tool in understanding the expression of key genes, both in the free-living and bacteroid state. Expression of the fix genes occurs within nodules, but not in the free-living state. *nifA* is expressed in the free-living state though its protein does not function under free-living conditions.
- The lux tool can be used to validate predictions concerning transcription start sites and essential sites upstream of a gene of interest. Control of *nifA* looks to come from a region within the *fixX* gene.

This work has been carried out as part of a BBSRC-funded Doctoral Training Grant. Thanks go to the Microbiology Society for providing a travel grant to present this work
We would like to thank Allan Downie and Anne Edwards for development of the lux bioreporter system.

References:

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