Divalent Metal Transport Systems Required by *Rhizobium leguminosarum* bv. *viciae*

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Summary

- *Rhizobium leguminosarum* bv. *viciae* 3841 (Rlv3841) requires both Mn^{2+} and Mg^{2+} for effective symbiosis.
- Δ*sitA mntH* is Fix^- on pea and on *Vicia faba*.
- Δ*mgtE* is Fix^- on pea but Fix^+ on *V. faba*.

Manganese (Mn^{2+})

Rlv3841 utilises two types of Mn^{2+} transporters:

1) **SitABCD**. ABC transporter encoded by RL3884-87.
2) **MntH.** NRAMP (*N*atural *R*esistance *A*ssociated *M*acrophage *P*rotein) encoded by RL0940.

Both are transcriptionally upregulated in early bacteroids compared to free-living cells.

- When both systems are absent (Δ*sitA mntH*) high Mn^{2+} supplementation is required for growth.
- Expression of *sitA-gus* and *mntH-gus* is upregulated under Mn^{2+} limitation **Figure 1**.
- Regulation is dependent on *m*anganese *u*ptake *r*egulator Mur (originally characterised by Díaz-Mireles *et al.,* 2004) **Figure 1**.

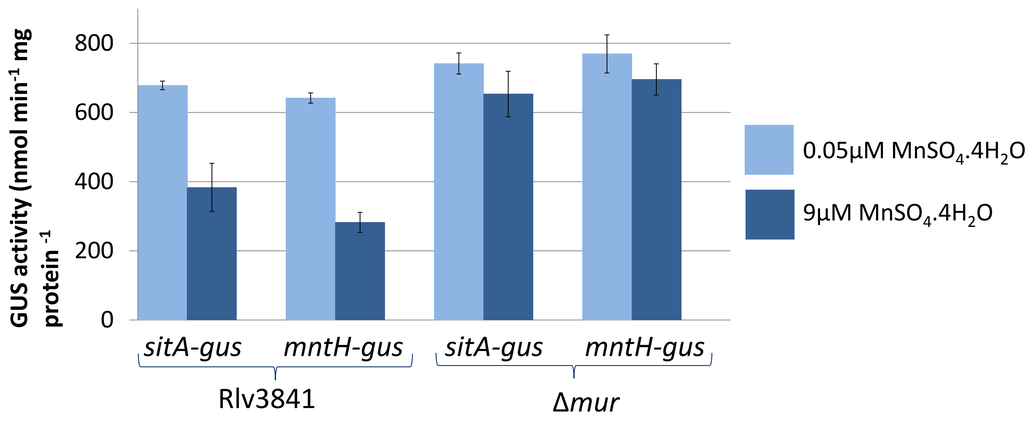


Figure 1. Expression of *sitA-gus* and *mntH-gus* as determined by GUS activity.

SYMBIOTIC PHENOTYPE

- Δ*sitA* and Δ*mntH* are both **Fix^+** on pea and *V. faba*.
- Δ*sitA mntH* is **Fix^-** on pea and *V. faba* **Figures 2 and 3**.
- Light microscopy and TEMs of nodule sections show plant cells devoid or sparsely packed with bacteroids for Δ*sitA mntH* –inoculated plants **Figure 4**.

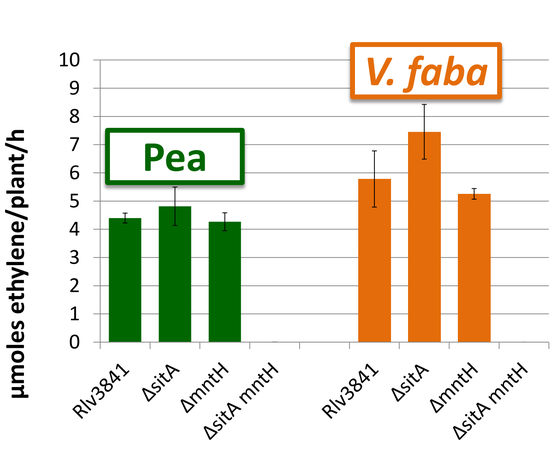


Figure 2. Rates of N_2 fixation as determined by acetylene reduction.

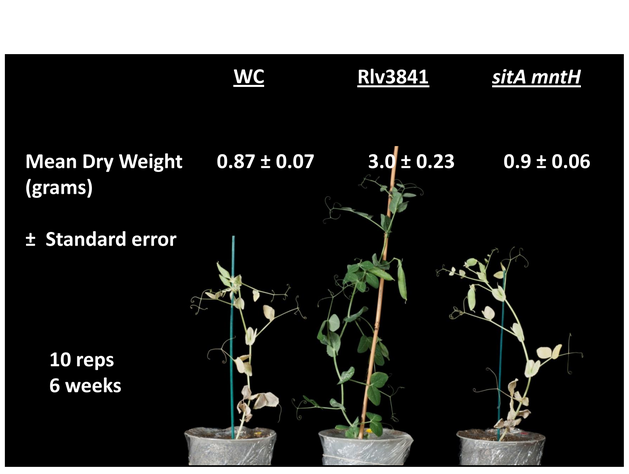


Figure 3. Photos and dry weights of six-week pea inoculated with Rlv3841 and Δ *sitA mntH*.

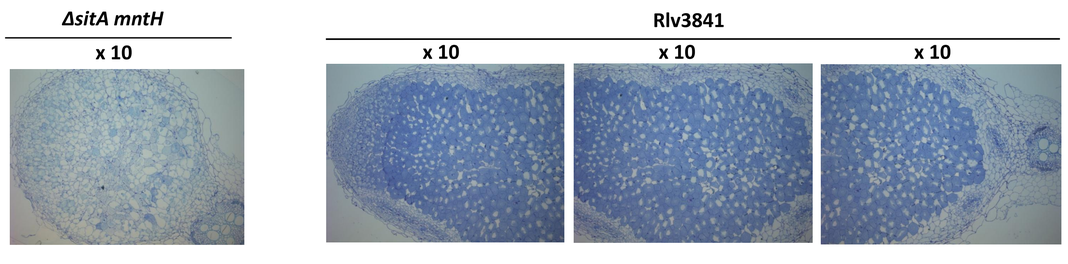
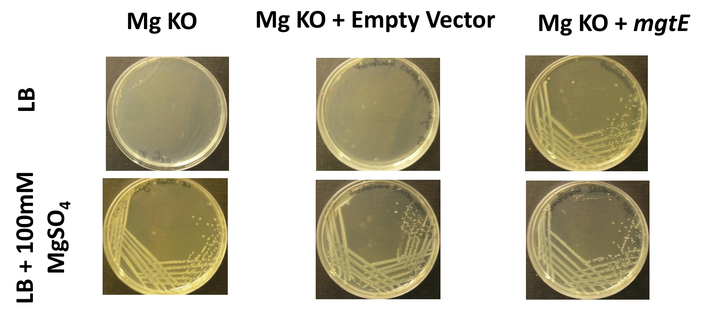


Figure 4. Nodule sections from pea inoculated with Δ *sitA mntH* (Fix^-) and compared to Rlv3841 inoculated (Fix^+) .

Magnesium (Mg^{2+})

- Gene RL1461 encodes a putative **MgtE** transporter associated with Mg^{2+} uptake.
- A long lag phase is seen when this gene is inactivated (Δ*mgtE*).
- RL1461 can complement an *Escherichia coli* strain (Hattori, M *et al*., 2009) that is auxotrophic for Mg^{2+} uptake (Mg KO) **Figure 5**.

Figure 5. Complementation of a *E. coli* strain auxotrophic for Mg^{2+} uptake with *mgtE* (RL1461) from Rlv3841.



SYMBIOTIC PHENOTYPE

- On **pea**, Δ*mgtE* is severely **defected in its ability to fix N_2** **Figures 6 and 7** but is able to infect plant cells and form branched-shaped bacteroids.
- On ***V. faba*** a **Fix^+** phenotype was observed **Figure 6**.
- Metal transporters required by a *Rhizobium* strain for effective symbiosis **differ between legume hosts.**

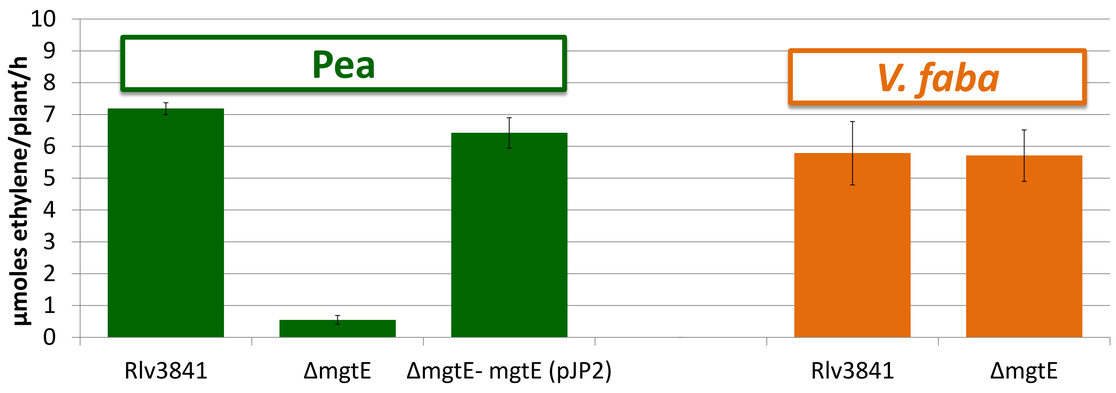


Figure 6. Rates of N_2 fixation as determined by acetylene reduction.

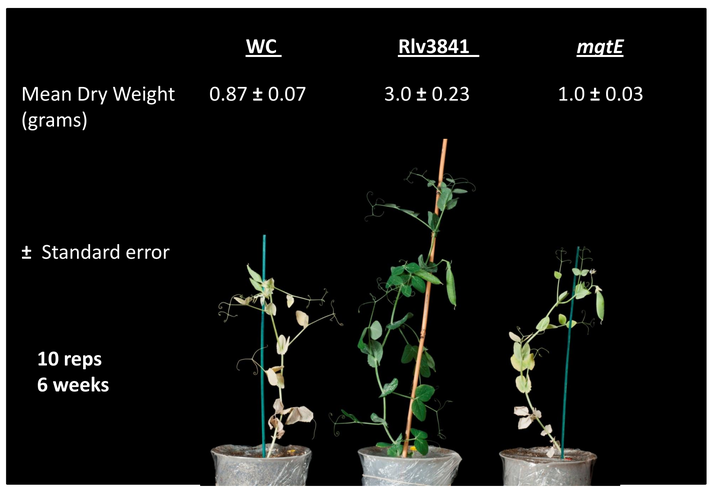


Figure 7. Photos and dry weights of six-week pea inoculated with Rlv3841 and Δ *mgtE*.

Future Directions

- **Atomic Absorption Spectroscopy** to determine concentrations of the two metals in pea and *V. faba* nodules.
- Investigate requirement of Mn^{2+} by *R. leguminosarum* bv. *viciae* and *R. leguminosarum* bv. *phaseoli* in **indeterminate** and **determinate** nodules (respectively).

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