Genetic determinants of suboptimal N_2 fixation and host range in the *Medicago-Sinorhizobium* symbiosis

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Background

- The *Medicago* genus is one of prime importance to sustainable agriculture due to the ability of its members to fix atmospheric N_2 in symbiosis with bacteria.
- Although two species of bacteria - *Sinorhizobium meliloti* and *S. medicae* – can form symbiotic interactions with *Medicago* spp., the host range exhibited by these microsymbionts varies (Figure 1):
  - *S. meliloti is* Nod^- on *M. murex* and Nod^+Fix^- on *M. polymorpha*
  - *S. medicae* is Nod^+Fix^+ on both these hosts
- Recently the complete genome sequence of *S. medicae* WSM419 was reported[1] , complementing that of the widely studied 'lab workhorse' *S. meliloti* 1021 (Sm1021), making these two strains key references for study.
- While both Sm1021 and WSM419 are able to nodulate and fix N_2 on the model legume *M. truncatula* A17, only WSM419 is able to do so effectively under N-limited conditions[2] (Figure 1).
- The genetic determinants of these variations in N_2 fixation effectiveness and host range are not known.
- Therefore, we propose to investigate the basis of the effectiveness and host range differences of these strains using a cosmid library approach.

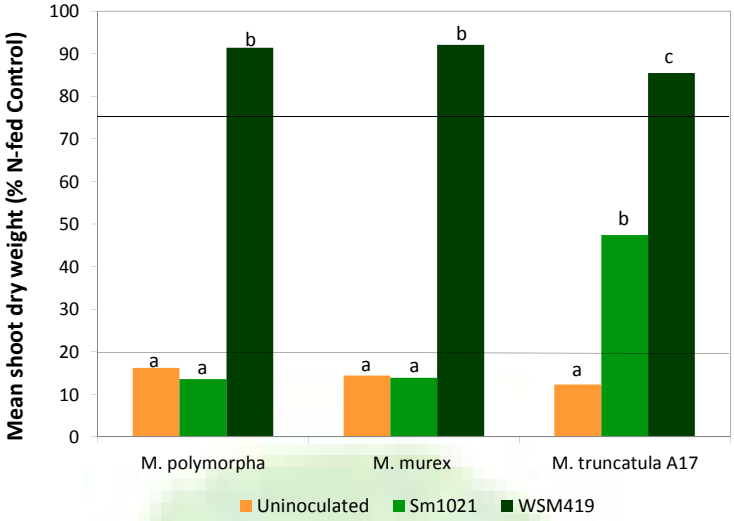

Figure 1 - Mean shoot dry weight of three *Medicago* hosts grown in sterile sand for 42 days with nutrients in N-free conditions and either uninoculated, or inoculated separately with Sm1021 or WSM419. Bars which share a letter for a given plant host are not significantly different (P<0.05).

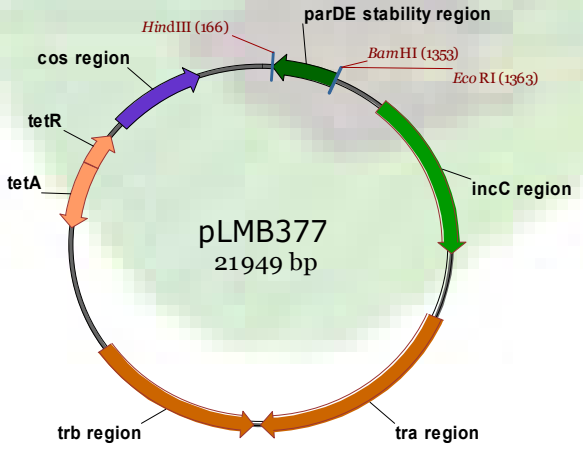

Figure 2 - Map of the cosmid pLMB377, carrying the cos region for packaging into λ-phage, the parDE stability region, *tetA* and *tetR* encoding tetracycline resistance, as well as transfer functions (*tra* and *trb* regions).

Cosmid Library Construction

- The cosmid pLAFR3, containing the *cos* sites for packaging into λ-phage and transduction of *E. coli,* was selected as a starting point.
- Although pLAFR3 carries the *incC* plasmid stability region, work in our lab has suggested the cosmid is not reliably stable *in planta*.
- Therefore, the *parDE* region was PCR amplified from pJP2 and cloned into pLAFR3 as a *Hind*III-*Bam*H1 fragment, creating pLMB377 (Figure 2).
- *parDE* encodes an antioxin-toxin protein pair, with demonstrated stability *in planta* on *Pisum sativum*[3] and *M. sativa*[4].
- The stability of pLMB377 *in planta* is currently being confirmed with Sm1021 (pLMB377) on *M. truncatula* A17.
- pLMB377 will then be used to construct a cosmid library of *S. medicae* WSM419 (partial *Eco*R1 digest of gDNA) and cloned into *Eco*R1-digested pLMB377.
- Based on the known genome size of WSM419 (6.8 Mbp), will require approximately 1,300 colonies for 99% genome coverage.
- Mobilise the WSM419 cosmid library into a Sm1021 background.

Screening for effectiveness and host range determinants

- Screen Sm1021 carrying the WSM419 cosmid library on *M. polymorpha*, *M. murex* and *M. truncatula*.
- Look for large pink nodules, indicative of an effective interaction.
- Should be straight-forward for *M. polymorpha* and *M. murex* interactions, as wild-type Sm1021 is Fix^- and Nod^- on these hosts, respectively.
- The situation is more complicated for *M. truncatula* A17; Sm1021 is Fix^+ on this host, albeit only partially (Figure 1).
- But, nodulation of *M. truncatula* A17 by Sm1021 compared to WSM419 is very different - Sm1021 yields more numerous nodules which are paler, smaller in size and more widely distributed over the root system than those induced by WSM419 on this host (Figure 3).
- Therefore, it will be necessary to screen for a change in nodule morphology on *M. truncatula.*
- Successful putative clones will be isolated from nodules, the symbiotic phenotype will be authenticated and the clone(s) sequenced.

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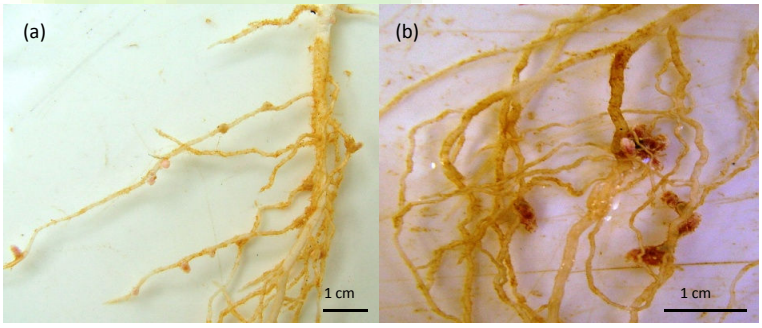

Figure 3 - Photographs of *M. truncatula* A17 roots inoculated with (a) Sm1021 and (b) WSM419 and harvested 42 days post-inoculation.

References

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2. Terpolilli *et al.* (2008) *New Phytol.* 179: 62-6
3. Prell *et al.* (2009) *P.N.A.S.* 106: 12477-82
4. Weinstein *et al.* (1992) *J. Bact.* 174: 7486-9

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