Rapid report

The model legume Medicago truncatula A17 is poorly matched for N2 fixation with the sequenced microsymbiont Sinorhizobium meliloti 1021

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Summary

- Medicago truncatula (barrel medic) A17 is currently being sequenced as a model legume, complementing the sequenced root nodule bacterial strain Sinorhizobium meliloti 1021 (Sm1021). In this study, the effectiveness of the Sm1021–M. truncatula symbiosis at fixing N2 was evaluated.
- N2 fixation effectiveness was examined with eight Medicago species and three accessions of M. truncatula with Sm1021 and two other Sinorhizobium strains. Plant shoot dry weights, plant nitrogen content and nodule distribution, morphology and number were analysed.
- Compared with nitrogen-fed controls, Sm1021 was ineffective or partially effective on all hosts tested (excluding M. sativa), as measured by reduced dry weights and shoot N content. Against an effective strain, Sm1021 on M. truncatula accessions produced more nodules, which were small, pale, more widely distributed on the root system and with fewer infected cells.
- The Sm1021–M. truncatula symbiosis is poorly matched for N2 fixation and the strain could possess broader N2 fixation deficiencies. A possible origin for this reduction in effectiveness is discussed. An alternative sequenced strain, effective at N2 fixation on M. truncatula A17, is Sinorhizobium medicae WSM419.


Introduction

The Medicago genus is one of prime importance to agriculture because of the ability of its members to fix atmospheric N2 in symbiosis with bacteria. Medicago sativa (alfalfa, lucerne), arguably the most widely cultivated species, has been studied extensively, often in conjunction with the microsymbiont Sinorhizobium meliloti 1021 (Sm1021). The sequencing of the genome of Sm1021 (Galibert et al., 2001) highlighted a dearth of knowledge of host genetic determinants, making sequencing of the host genome a necessary next step. Medicago sativa was poorly suited to this role and the more...
Amenable Medicago truncatula (barrel medic) was chosen (Barker et al., 1990; Cook, 1999). The Sm1021–M. truncatula symbiosis has now emerged as the model system for studying indeterminate nodulation, and the sequencing of the M. truncatula genome continues (Young et al., 2005).

A broad range of effectiveness of symbiotic interactions exists in legume symbioses (Sprent, 2007). Howieson et al. (2005) have classified these interactions into four groups.

- No symbiotic interaction, where no infection of the host occurs.
- A parasitic interaction where nodule-like bodies form, but no N₂ is fixed.
- A partially effective symbiosis where fixation occurs, but at a rate between 20 and 75% of the nitrogen (N)-fed control.
- An effective symbiosis where biomass and N accumulation occur at levels 75% or higher, relative to the N-fed control.

At present, there are few published data quantifying N₂ fixation between the model host M. truncatula and Sm1021. However, data are available for S. meliloti SU47 (Brockwell & Hely, 1966; Snyman & Strijdom, 1980), the parent strain of Sm1021 (Meade et al., 1982). Here, we provide evidence that the symbiosis between the model legume M. truncatula and the sequenced strain Sm1021 is poorly matched for N₂ fixation.

**Materials and methods**

**Bacterial strains and plant accessions**

Sinorhizobium meliloti 1021 was obtained from Professor Sharon Long (Stanford University, USA). Sinorhizobium medicae WSM1021 (Centre for Rhizobium Studies WSM Genebank) is an isolate from Sardinia (Italy; Howieson & Ewing, 1986) and was the commercial inoculant for annual Medicago spp. in Australia from 1985 to 1993 (Bullard et al., 2005). Sinorhizobium meliloti WSM1022 is a field isolate obtained from Naxos (Greece) from the annual legume Medicago orbicularis (J. G. Howieson, CRS, WSM Genebank).

Medicago truncatula accessions SA1619 (cv. Jemalong), SA27783 (cv. Caliph) and SA37443 (A17, the reference line for the genome sequencing effort), Medicago arabica SA36043, Medicago littoralis SA421 and Medicago tornata SA3639 were obtained from the Genetic Resource Centre, SARDI (South Australian Research and Development Institute, Adelaide, South Australia). Medicago sativa cv. Sceptre, Medicago polymorpha cv. Santiago, Medicago murex cv. Zodiac and Medicago sphaerocarpus cv. Orion, were obtained from the Department of Agriculture and Food, Western Australia.

**Experiments to assess N₂ fixation**

Plants were grown in free-draining pots in a 1:1 mix of yellow sand and washed river sand, as described by Howieson et al. (1995), where growth of legumes is limited by N deficiency, except when they are effectively nodulated. Seeds were scarified, surfaced sterilized in 70% (v/v) ethanol (1 min) and 4% (w/v) NaOCl (3 min), rinsed six times in sterile water and placed onto 0.9% (w/v) water agar plates to germinate. When radicles had emerged, six seedlings were sown aseptically into each pot. Inoculant bacteria were cultured on half lupin-agar (Howieson et al., 1988) for 3 d at 28°C. Cultures were suspended in 1% (w/v) sucrose solution at 10⁶ cells ml⁻¹ and 1 ml of this suspension was inoculated onto each seedling at sowing. Plants were thinned to four per pot after 2 wk.

Control plants were grown in identical conditions but not inoculated. Plus N controls were fed weekly with 5 ml of 0.1 M KNO₃ and negative controls received no N. All plants were given regular nutrients and sterile water as required (Howieson et al., 1995). Three replicates were prepared for each treatment and, within each experiment pot, position was randomly allocated.

Plant shoots were excised 42 d postinoculation and dried for 2 d at 60°C, then weighed. Roots were carefully washed free of soil and were assessed for nodule number, distribution and morphology. Nodules were excised, sectioned, stained and examined as previously described (Yates et al., 2007). Effective, partially effective and ineffective symbioses were determined using the criteria established by Howieson et al. (2005). Shoot N content was determined on a Leco F528 Nitrogen Analyzer (CSBP Soil and Plant Laboratory, Perth, Australia).

Experiment 1 investigated the potential for N₂ fixation with three strains of Sinorhizobium (WSM419, WSM1022 and Sm1021) inoculated separately onto five annual species of Medicago. Experiment 2 investigated N₂ fixation with Sm1021 and WSM1022 on three M. truncatula accessions (SA1619, SA27783 and SA37443), as well as the closely related M. littoralis and M. tornata. Experiment 3 investigated N₂ fixation and nodule morphology produced by three strains of Sinorhizobium inoculated separately onto the perennial medic M. sativa in comparison with M. truncatula SA37443.

**Statistical analysis**

Experiments were analysed using the analysis of variance (ANOVA) package of genstat version 9. For each plant species/accession, significant differences between treatments were determined by one-way ANOVA followed by a post hoc Fisher’s least significant difference (LSD) test with P < 0.05. Data for experiments 1 and 2 are presented as a percentage of the N-fed control and were log₁₀ transformed before the statistical analysis.

**Results**

N₂ fixation across a broad range of Medicago hosts

The effectiveness of Sm1021, WSM1022 and WSM419 on a selection of Medicago hosts was assessed through measurement of plant production (dry weight of shoots) in a growth environment limited by available N. WSM1022 and WSM419
inoculated onto *M. truncatula* out-yielded the Sm1021–*M. truncatula* symbiosis by more than 50% (*P* < 0.05). The two former strains were effective with the model host whereas Sm1021 achieved only partial effectiveness (Fig. 1). Sm1021 and *S. meliloti* WSM1022 did not differ in symbiotic characteristics with four other hosts, with both strains failing to fix N\(_2\) on *M. polymorpha*, *M. murex* and *M. arabica*, whilst fixing very poorly on *Medicago sphaerocarpus* (Fig. 1). By contrast, *S. medicae* WSM419 was either effective (*M. polymorpha*, *M. murex*) or partially effective (*M. sphaerocarpus*, *M. arabica*) on these same hosts (Fig. 1).

The partial effectiveness displayed by Sm1021 on *M. truncatula* was also evident from a broader comparison of other compatible hosts (Fig. 2). Whereas WSM1022 fixed effectively with hosts *M. littoralis* and *M. tornata*, as well as with two additional accessions of *M. truncatula*, Sm1021 was suboptimal for N\(_2\) fixation across these hosts (Fig. 2).

Plant shoot N content revealed that N\(_2\) fixation by Sm1021, WSM1022 and WSM419 was not significantly different on *M. sativa* (Fig. 3). Consistent with experiments 1 and 2, Sm1021 on *M. truncatula* showed a significantly lower (*P* < 0.05) shoot N content than either WSM1022 or WSM419 (Fig. 3). Analysis of the plant dry weights (data not shown) confirmed the shoot N-content data.

**Nodule development**

Average nodule numbers on *M. sativa* inoculated separately with strains Sm1021, WSM419 or WSM1022 did not differ significantly, ranging from five to eight per plant. These nodules were relatively large, dark pink and tightly distributed in the upper root zone (Fig. 4). On *M. truncatula*, nodule number, distribution and morphology were not different when inoculated separately with either WSM1022 or WSM419. By contrast, *M. truncatula* inoculated with Sm1021 showed greater numbers of nodules (*P* < 0.05), ranging from 15–18 per plant. These nodules were smaller
and paler, distributed across the entire root system and green at the proximal end (Fig. 4).

Nodule sections revealed that although plant cells did contain bacteroids, there were fewer plant cells with bacteroids in the Sm1021–M. truncatula symbiosis than in M. truncatula inoculated with either WSM1022 or WSM419. Starch granules were also evident at the plant cell periphery as was a marked occlusion of the interstitial spaces (data not shown). These phenomena were not visible in any effective symbiotic partnership examined.

Discussion

There has been widespread study of the Sm1021 symbiosis with M. sativa, leading to an accumulation of genetic, symbiotic and biochemical data on these organisms and culminating in the genome sequencing of Sm1021 (Galibert et al., 2001). Medicago sativa was rejected for sequencing, in part because of its large genome, tetraploidy and out-crossing requirement, and M. truncatula was chosen instead (Barker et al., 1990; Cook, 1999). Recent research has indicated that the accession selected (A17) carries a chromosomal translocation, which results in the semisterility of pollen in hybrids (Kamphuis et al., 2007). The effect on symbiotic performance is unknown.

We know of no published data on the effectiveness of M. truncatula with Sm1021 at N₂ fixation. Simsek et al. (2007) have noted that this symbiosis ‘was much less efficient ... than the other compatible strains’, an observation supported by Garau and co-workers (2005). However, data are available showing the effectiveness of M. truncatula with the parent strain SU47 (Brockwell & Hely, 1966; Snyman & Strijdom, 1980); both papers report the symbiosis to be relatively effective, although only Brockwell & Hely (1966) have compared dry weights with an N-fed control.

In the present study, plant dry weight, N content and nodule data together provide strong evidence for a poorly matched symbiosis between Sm1021 and M. truncatula. The larger number of nodules on the roots of M. truncatula and their atypical morphology are indicative of ineffective nodulation (Mishustin & Shil’nikova, 1971; Frederick, 1978); cobalt and molybdenum deficiency, both of which compromise N₂ fixation, also produce high nodule numbers (Anderson & Spencer, 1950; Riley & Dilworth, 1982). The lower dry weights produced by Sm1021 on M. littoralis and M. tornata relative to WSM1022 suggest that there may be
broader symbiotic deficiencies in the former strain. That Sm1021 fixed more effectively on M. sattiva reflects the historical utilization of its parent SU47 as a commercial inoculant with M. sattiva (Bullard et al., 2005) and the common use thereafter of Sm1021 in the laboratory.

Why is it that SU47 appears to be more effective on M. truncatula than Sm1021? SU47, first isolated in 1939 (Vincent, 1941) from M. sattiva growing at Bathurst, Australia, has, over the years, been sent to many research laboratories around the world and presumably cultured and stored under varying conditions. It is difficult to say how similar the parent strain isolated 62 yr ago is to the spontaneous StrR strain Sm1021 (Meade et al., 1982) sequenced in 2001 (Galibert et al., 2001), but it is possible that the strain acquired altered symbiotic characteristics in the intervening years. The difference in calcium-spiking behaviour between SU47 derivatives Sm1021 and Sm2011 (Wais et al., 2002) with M. truncatula root hairs may also be the result of long-term cultivation.

Researchers working on legumes need to be aware that the Sm1021–M. truncatula A17 symbiosis is not optimally matched for N2 fixation. Molecular analyses of symbiotic requirements need a reliable benchmark against which to make comparisons. The poor effectiveness of the Sm1021–M. truncatula symbiosis suggests that it may not be able to fulfil this role. The recently sequenced S. medicate WSM419 (Genbank accession NC_009636), shown here to be a better microsymbiont for M. truncatula than Sm1021, offers those studying host–strain interactions the opportunity to work on a highly effective symbiosis.

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References


