

CAMBIA

Transbacter Import Information

### **Bacterium background**

Rhizobia, is a collective name of the genera *Rhizobium*, *Sinorhizobium*, *Mesorhizobium* and *Bradyrhizobium*, which are soil and rhizosphere bacteria of agronomic importance because they perform nitrogen-fixing symbioses with leguminous plants. *Rhizobium* and *Sinorhizobium* are in the family Rhizobiaceae, while *Mesorhizobium* and *Bradyrhizobium* members of Phyllobacteriaceae and Bradyrhizobiaceae, respectively.

*Sinorhizobium meliloti*, is a common Gram-negative soil and rhizosphere bacterium, best known for its ability to induce the formation of nodules on the roots of *Medicago*, *Melilotus* and *Trigonella* sp. Inside the nodules, differentiated bacteria called bacteroids fix atmospheric nitrogen (*ie* reduce N<sub>2</sub> into NH<sub>3</sub>) to the benefit of the plant. The *S. meliloti* genome contains three replicons: a 3.65-Mb chromosome and two megaplasmids, pSyma (1.35 Mb) and pSymb (1.68 Mb). *Sinorhizobium meliloti* strain 1021 was provided to CAMBIA from the Australian National University Collection (Canberra, Australia).

The strain *Rhizobium* sp. NGR234, is another Gram-negative soil and rhizosphere bacterium, which is known to live in symbiosis with more than 110 genera of legumes as well as the non-legume *Parasponia andersoni*. *Rhizobium* sp. NGR234 has a chromosome 3.5 Mb in length, a megaplasmid of more than 2 Mb (pNGR234b), and a smaller plasmid 536,165 bp in length (pNGR234a) that carries most of the genes used for symbioses with legumes. The 536,165-bp sequence of pNGR234a has now been completely analysed and released to the public domain. *Rhizobium* sp. NGR234 was provided to CAMBIA from the Australian National University Collection (Canberra, Australia).

*Mesorhizobium loti* is a member of rhizobia which is able to form determinant-type globular nodules and perform nitrogen-fixation on several *Lotus* species. The genome of *M. loti* consisted of a single chromosome (7,036,071 bp) and two plasmids, designated as pMLa (351,911 bp) and pMLb (208,315 bp). *Mesorhizobium loti* MAFF303099 was obtained from University of Otago, (Dunedin, NZ).

### **Ti Plasmid construction:**

The modified Ti plasmids, were created using a suicide vector which was constructed by T/A cloning of a PCR-amplified fragment from the Ti plasmid of EHA105 into TOPO vector pCR2.1 (Invitrogen, CA) corresponding to 1316 bp encompassing the *virG* gene (pTiWB1) or a 995 bp part of the non-essential gene *moaA* (pTiWB3). The *oriT* fragment was cloned in this vector as an *Xba*I fragment following amplification from pSUP202. Electroporation of the suicide vector into EHA105 resulted in integration of the whole vector by a single crossing-over event, thereby creating two functional *virG* genes (pTiWB1) or insertion of a second truncated *moaA* gene. Co-integration was confirmed by PCR across the integration site and by Southern blotting showing duplication of the target locus. The modified Ti plasmids were constructed at CAMBIA (Canberra, Australia).

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#### **pCAMBIA1105.1R construction:**

The binary vector pCAMBIA1105.1R, containing hygromycin phosphotransferase (*aph* or *Hyg<sup>R</sup>*) and *GUSPlus* genes for expression in plants, was derived from pCAMBIA1305.1 (GenBank no. AF354045) by insertion of a spectinomycin/streptomycin marker into the *SacII* site and the removal of the kanamycin marker. The spectinomycin/streptomycin marker was generated by PCR amplification from pPZP200. In addition, the *PvuII*-*PvuII* MCS fragment in pCAMBIA1305.1 was replaced by the 99bp larger *PvuII*-*PvuII* MCS from pCR2.1. The binary vector was constructed at CAMBIA (Canberra, Australia).

#### **Bacterial strain construction:**

The modified Ti plasmid (pTiWB3) was mobilised to *Sinorhizobium meliloti* in a triparental mating with EHA105 (pTiWB3), and *E. coli* helper strain RP4-4 (carbenicillin and tetracycline resistance). The binary vector, pCAMBIA1105.1R, was then introduced by electroporation. The bacterial strain containing pTiWB3 and the binary vector pCAMBIA1105.1R was constructed at CAMBIA (Canberra, Australia).

#### **Differences between the modified and unmodified bacterium:**

The modified bacterium, containing the Ti plasmid and the binary vector pCAMBIA1105.1R, is able transfer a portion of DNA (called T-DNA) from the binary vector into a plant cell. Once in the plant cell, the T-DNA targets the nucleus, allowing for integration into the plant genome and expression of genes encoded on the T-DNA. The unmodified bacterium is unable to transfer DNA to a plant host.

**Shipping and safeguards against escape or dissemination:** Bacterial stabs will be made onto YM media in a 2 ml polypropylene, screw top vial, the vial will be placed in a flat clear plastic holder and shipped in a bubble-wrap envelope. Envelope size approximately 15 x 23 cm.