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_2 into NH3) 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. NGR234was 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*^{R}) and *GUSPlus* genes for expression in plants, was derived from pCAMBIA1305.1 (GenBank no. AF354045) by insertion of a spectinomycin/streptomycin marker into the *Sac*I site and the removal of the kanamycin marker. The spectinomycin/streptomycin marker was generated by PCR amplification from pPZP200. In addition, the *Pvu*II-*Pvu*II MCS fragment in pCAMBIA1305.1 was replaced by the 99bp larger *Pvu*II-*Pvu*II 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.