Sequences of genotyping primers and sizes of PCR products

Bacterial strains were genotyped by PCR using the following primer sets for the chromosomes and megaplasmids.

Genotyping of bacterial strains

Bacterial species	Selective growth condition (antibiotic concentration in µg/ml)	Genotyping by PCR	Size of PCR product (bp)
Rhizobium sp. NGR234 strain ANU240	YM + Streptomycin (200)	Sme16SrRNA (chromos.) NodD1-NGR234 (pSym)	820 350
Sinorhizobium meliloti 1021	YM + Streptomycin (200)	Sme16SrRNA (chromos.) NodQ (pSymA) NodQ2 (pExo)	820 420 360
Mesorhizobium loti strain MAFF303099	YM + Gentamycin (20)	Mlo16SrRNA (chromos.) RepCa (pMLa) RepCb (pMLb)	500 200 320
A. tumefaciens EHA105	LB + Rifampicin (20)	Atu16SrRNA (chromos.) AttScirc (circular chromos.) VirJ (Ti plasmid) MoaA (Ti plasmid) AttS-pAT (pAT megaplasmid)	410 436 948 433 436

Sequence of primers used to distinguish bacterial strains

Bacterial strain	Genome location	Targeted gene	Sequence 5'-3'
Rhizobium sp. NGR234	Chromosomal	16SrDNA[1]	TGTGCTAATACCGTATGAGC
			CAGCCGAACTGAAGGATACG
	pSym	*NodD1*	GCCAGAAATGTTCATGTCGCACA
			AATGGGTTGCGGAAGTTCGGT
S. meliloti 1021	Chromosomal	16SrDNA[1]	TGTGCTAATACCGTATGAGC
			CAGCCGAACTGAAGGATACG
	pSym	*NodQ*	GACAGGATCCTCCACGCTCA
			CGCCAGGTCGTTCGGTTGG
	pExo	*NodQ2*	GACAGGATCCTCCACGCTCA
			GCTCATAGGGCGAGGATACA
M. loti MAFF303099	Chromosomal	16SrDNA	CCCATCTCTACGGAACAACT
			ACTCACCTCTTCCGGACTCG
	pMLa	*RepC*	GACGGCCGAGCCAAGGACGA
			CACATGGCAAGCCTCCTCA
	pMlb	*RepC*	GATGCTGGAAAGCTTCACAAGT
			CACATGGCAAGCCTCCTCA
A. tumefaciens EHA105	Chromosomal	16SrDNA	GAATAGCTCTGGGAAACTGGAAT
			CGGGGCTTCTTCTCCGACT
	Chromosomal	*attS*circ	CAGGCTCAAACCGCATTTCC
			GTAAGTCCAGCCTCTTTCTCA
	pAT	*attS*(pAT)	GTGCTTCGGATCGACGAAAC
			GGAGAATGGGAGTGACCTGA
	pTi	*VirJ*[2]	TGACCTTGGCCAGGGAATTG
			TCCTGTCATTGGCGTCAGTT
		MoaA[2]	CTCCCAAGAGGGTCGTTGAC
			ATGGATCCTGCCGTGGTCTCGTGTTCT GG

[1] These primers amplify the 16S rDNA genes from both *S. meliloti* and *Rhizobium* sp. NGR234

[2] Location on the pTiBo542 plasmid map at 8 o'clock and 4 o'clock for *VirJ* and *MoaA* respectively

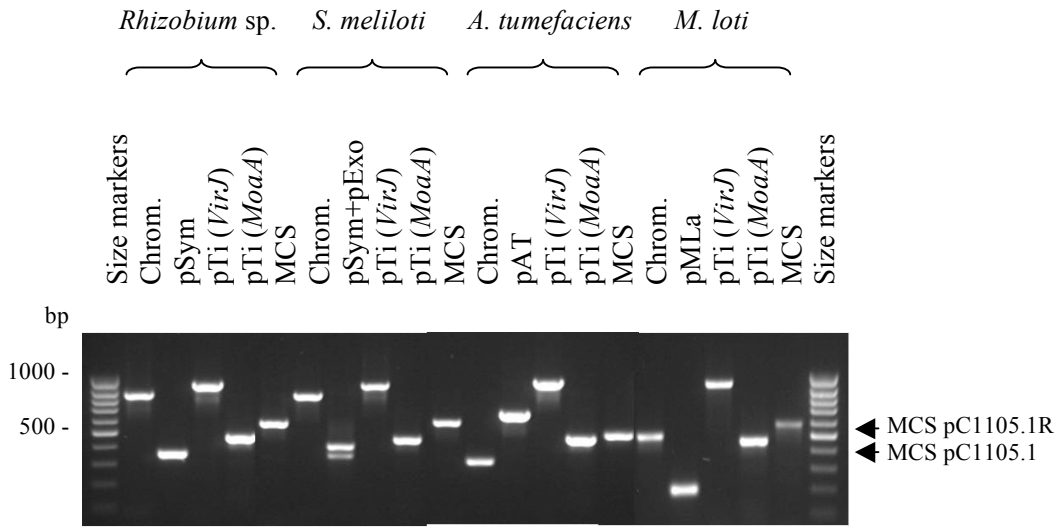


Figure shows genotyping of gene transfer competent bacterial strains containing pTiWB3 by selective PCR amplification of chromosomal and megaplasmid markers, and the differently sized MCS in pCAMBIA1105.1R (pC1105.1R) and pCAMBIA1105.1 (pC1105.1) vectors (arrows). Figure from Broothaerts *et al.*, 2005 Nature.