Incompatibility behavior of a symbiotic plasmid pMH7653Rb in *Mesorhizobium huakuii* 7653R

HU GuoYuan1*, LI WeiWei1 & ZHOU JunChu2

1Key Laboratory for Green Chemical Process of Ministry of Education, Wuhan Institute of Technology, Wuhan 430073, China; 2State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China

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*Mesorhizobium huakuii* strain 7653R harbored two indigenous plasmids named pMH7653Ra and pMH7653Rb. The larger plasmid pMH7653Rb (symbiotic plasmid) was transferred to *M. huakuii* HN308SR harboring three plasmids: pMHHN308a, pMHHN308b and pMHHN308c, and HN3015SR harboring three plasmids: pMHHN3015a, pMHHN3015b and pMHHN3015c by tri-parent mating. Two stable indigenous plasmids, pMHHN308b and pMHHN308c of HN308SR, were co-eliminated due to the introduction of pMH7653Rb, and the transconjugant was named HN308SRN14. The results implied that pMH7653Rb and pMHHN308b, pMHHN308c were incompatible and might have been ascribed to the same incompatible group. The plasmid profiles of transconjugant HN3015SRN14 showed that the second largest plasmid pMHHN3015b of HN3015SR was cured due to the introduction of pMH7653Rb. The results also implied that pMH7653Rb and pMHHN3015b were incompatible. Results from plant nodulation tests showed that pMH7653Rb could only maintain the nodulation ability in transconjugant HN308SRN14 and its nodule number was more than that of wild strain HN308SR, but could not replace the nitrogen fixation effect of pMHHN308b and pMHHN308c. The plasmid cured mutant HN308SRN14D harboring only pMHHN308a formed null nodules that demonstrated pMHHN308a was relevant to nodulation ability. HN3015SRN14 harboring pMH7653Rb, pMHHN3015a and pMHHN3015c formed null nodules while HN3015SRN14D containing pMHHN3015a and pMHHN3015c lost the nodulation ability. The plasmid replication repC-like gene sequences were detected by a polymerase chain reaction from 7653R, HN308, HN3015, HN308SRN14 and HN3015SRN14. The repC gene sequence similarities of the strains tested attained 99%.

*Astragalus sinicus*, conjugative plasmid transfer, indigenous plasmid, *Mesorhizobium huakuii*, plasmid curing, plasmid incompatibility, Tn5-sacB


Incompatibility or the incapacity to coexist of two plasmids as independent replicons in the same cell results from the interference of the replication and/or partitioning functions between them [1]. The biological effects of plasmid incompatibility have been reported between rhizobia or between rhizobia and agrobacteria [2,3]. Plant pathogenic Ti plasmids in *Agrobacterium tumefaciens* were also cured by using the incompatibility of pMGTrepI [4]. However, very little is known about plasmid incompatibility and its molecular basis in *Mesorhizobium*. Identification of the plasmid replication gene repABC has been extensively used to investigate the molecular mechanism of plasmid incompatibility among rhizobia [3,5–7]. The incompatibility groups are reported with different groups of replication genes [8]. Multiple incompatibility groups are necessary for stable maintenance in multi-component genomes [9]. To fully understand repABC replication and segregation machineries, it is essential to identify the chromosomal elements associated
Strains of *Mesorhizobium huakuii* nodulate only on *Astragalus sinicus*. Previous reports showed that *M. huakuii* 7653R harbored two large plasmids named pMH7653Ra (174 kb) and pMH7653Rb (318 kb). Both nod and fix genes were carried by the larger plasmid pMH7653Rb [10]. Incompatibility behavior of a megaplasmid pMHHN3015c in *M. huakuii* HN3015 has been reported between *M. huakuii* strains HN308 and 7653R-1 [11]. The symbiotic plasmid pMH7653Rb of *M. huakuii* strain 7653R was labeled by Tn5-mob-sacB insertion, and the mutant strain 7653RT14 labeled as its symbiotic plasmid was obtained [12]. To understand the plasmid incompatibility of the symbiotic plasmid pMH7653Rb between *M. huakuii* strains, a Tn5-sacB-labeled symbiotic plasmid pMH7653Rb was transferred into HN308SR (harboring pMHHN308a, 150 kb; pMHHN308b, 294 kb; pMHHN308c, 428 kb) or HN3015SR (harboring pMHHN3015a, 150 kb; pMHHN3015b 294 kb; pMHHN3015c, 390 kb) by tri-parent mating. Two transconjugants and their plasmid-cured derivatives were obtained. The results showed that two stable indigenous plasmids, pMHHN308c, pMHHN308b and pMHHN308c of HN308SR, were co-eliminated due to the introduction of pMH7653Rb, and that pMH7653Rb and pMHHN3015b were incompatible. The repC genes of strains tested were cloned and sequenced. The implications of these results are discussed.

1 Materials and methods

1.1 Bacterial strains and culture conditions

*M. huakuii* 7653RT14, HN308, HN3015 and *Escherichia coli* MM294 have been described by Hu et al. [11–13]. The culture conditions of the strains tested were used as described previously by Hu et al. [11]. Antibiotics were used in the following final concentrations (in μg ml⁻¹): kanamycin (Km), 50; streptomycin (Sm), 200; rifamycin (Rif), 20; spectinomycin (Spe), 50; ampicillin (Amp), 100.

1.2 Plasmid detection, transfer and curing

Large plasmids of *Mesorhizobium* strains were visualized by electrophoresis in 0.7% (W/V) agarose gel according to the modified Eckhardt procedure [11,14]. Plasmid transfer between two strains of *M. huakuii* was carried out as described by Hu et al. [11]. A plasmid curing experiment was carried out by using the sacB positive selection method as described by Hu et al. [11,13].

1.3 PCR amplification

The PCR amplification of the kan gene encoded by Tn5-sacB and repC-like gene sequences was performed as described by Hu et al. [11].

1.4 Plant nodulation tests

Plant nodulation tests were carried out by using the *Astragalus sinicus* cultivar Honghua 1 in double jars with sterile sand and a nitrogen-free plant nutrient solution [11]. Plants were scored after 6 weeks for root nodules, fresh shoots and dried weight. Nitrogenase activity of the nodules was also assayed [15].

1.5 Statistical analysis

The means comparison and standard deviation of data analysis were carried out by statistical software Statistical Package for Social Science (SPSS) 10.0. Analysis of significant differences was done according to Duncan’s multiple range test.

2 Results

2.1 Incompatibility among pMHHN308c, pMHHN308b and pMH7653Rb

The *M. huakuii* strain HN308SR is a spontaneous Sm⁻ and Rif⁻ mutant of wild type strain HN308 harboring three plasmids. HN308SR formed effective nodules on *Astragalus sinicus*. The plasmid pMH7653Rb labeled by Tn5-mob-sacB was transferred from 7653RT14 into HN308SR by tri-parental mating. Ten Km-resistant colonies of transconjugant HN308SRN14 were randomly picked from TY plates containing Sm, Rif and Km and investigated for their plasmid profiles by agarose gel electrophoresis. All of them had apparently lost their first largest plasmid pMHHN308c (Figure 1), probably due to incompatibility between pMHHN308c and pMH7653Rb. The transfer frequency of pMH7653Rb was 4.07×10⁻⁶. In addition, the electrophoretic mobility of pMH7653Rb and pMHHN308b was nearly the same. It was difficult to confirm that both plasmids were coexisting in HN308SRN14. To identify whether the second large plasmid pMHHN308b was simultaneously cured or not, transconjugant HN308SRN14 was inoculated on a TY plate containing Sm, Rif and Km and investigated for their plasmid profiles. It was demonstrated that all of them had lost pMHHN308b::Tn5-mob-sacB and pMHHN308b. The derivative with pMHHN308c and pMHHN308b cured from HN308SR was named HN308SRN14D. It was observed that pMHHN308b disappeared when pMH7653Rb::Tn5-mob-sacB was introduced into HN308SR (Figure 1). These results suggested that the two stable indigenous plasmids pMHHN308c and pMHHN308b were co-eliminated by the introduction of pMH7653Rb::Tn5-mob-sacB.

The results of plant pot nodulation tests showed that pMH7653Rb could only maintain the nodulation ability in transconjugant HN308SRN14 and its node number was more than that of HN308SR, but it could not replace the