GENE TRANSFER IN THE ENVIRONMENT PROMOTES THE RAPID EVOLUTION OF A DIVERSITY OF SUBOPTIMAL AND COMPETITIVE RHIZOBIA FOR *BISERRULA PELECINUS* L.

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The emergence of biodiversity in rhizobia after the introduction of exotic legumes and their respective rhizobia to new regions is a challenge for contemporary rhizobiology. *Biserrula pelecinus* L. is a pasture legume species that was introduced to Australia from the Mediterranean basin and which is having a substantial impact on agricultural productivity on acidic and sandy soils of Western Australia and New South Wales (Howieson et al., 2000). This deep-rooted plant is also valuable in reducing the development of dryland salinity. This legume is nodulated by a specific group of root-nodule bacteria that belongs to *Mesorhizobium* (Nandasena et al., 2001, 2007).

We have recently shown the evolution of diverse opportunistic rhizobia able to nodulate *B. pelecinus* following *in situ* transfer of symbiotic genes, located on a mobile symbiosis island, from an inoculant strain to other soil bacteria (Nandasena et al., 2006). A symbiosis island was first described in *M. loti* strain ICMP3153 (Sullivan and Ronson, 1998). Genomic islands could become inert and stabilised due to genomic rearrangements. Therefore, the aim of our current research is to investigate whether the current commercial inoculant strain for *B. pelecinus* (WSM1497) potentiated the development of a diversity of strains able to nodulate this legume via lateral transfer of the symbiotic island from WSM1497 to other soil bacteria.

Nodules from commercially grown *B. pelecinus* were collected (in 8/2005; 5–6 years after introduction and inoculation) from four different sites in Western Australia. The 387 pure cultures from nodule crushes (Table 1) were fingerprinted with the RPO1 PCR primer (Richardson et al., 1995) to show that only 50.1% of the nodules were occupied by WSM1497. These 193 isolates were authenticated on *B. pelecinus* cv. Casbah in a glasshouse experiment, when 184 nodulated this legume. Nodule occupancy by WSM1497 varied significantly between field sites, from >80% of the nodules collected from Brookton and Kondinin to <30% of the nodules from Karlgarin and Wickepin (Table 1). Furthermore, there were only a few dominant strains at the latter two sites. At Karlgarin, 20%

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of the nodules were occupied by a strain designated as type B, 25% of the nodules by another strain designated as type C, whereas only 22% of nodules were occupied by WSM1497, thus indicating that these recently evolved strains are highly competitive for nodulation of *B. pelecinus*.

Table 1. Nodule occupancy of commercially grown B. pelecinus collected from different fields in the WA wheat belt five or six years after inoculation with WSM1497.

Site	Total no of isolates	% WSM1497	% novel isolates	% non symbionts
Brookton	44	86.4	9.1	4.5
Kondinin	120	84.2	13.3	2.5
Karlgarin	120	21.7	77.5	0.8
Wickepin	103	28.2	68.9	2.9
Total	**387**	**50.1**	**47.5**	**2.3**

To maximize the value of *B. pelecinus* in farming systems, its N_2-fixing symbiosis must be maintained at the highest level of efficiency. Therefore, we tested the N_2-fixation efficiency of 53 randomly selected authenticated isolates (methods as Nandasena et al., 2004), all of which were found to be less effective than WSM1497. However, the N_2-fixation efficiency among these isolates ranged from no N_2 fixation (six isolates) to about 70% of that of the commercial inoculant. Fourteen of these 53 diverse isolates were randomly selected for the sequencing of *dnaK* to infer the phylogenetic relationships. All 14 strains clustered within *Mesorhizobium* and distantly to the Mediterranean *Biserrula* mesorhizobia. Furthermore, the symbiosis-island insertion regions of these 14 strains (Nandasena et al., 2006) had an identical sequences that was 100% similar to that of WSM1497, indicating a possible transfer from the commercial inoculant.

The results show the rapid evolution of competitive, yet suboptimal, strains for N_2 fixation on *B. pelecinus*, following the lateral transfer of a symbiosis island from the commercial inoculant to other soil bacteria. At present, we are constructing a genetically stable inoculant strain for *B. pelecinus* by inactivating the genes responsible for symbiosis-island transfer and thereby, reducing the chances of lateral gene transfer between the inoculant and soil bacteria in order to manage the future productivity of *B. pelecinus* at optimum levels.

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