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SYMBIOSIS

Breaking the rhizobial barrier

> “the barrier to a symbiotic lifestyle … can be overcome by selection for adaptive changes in the legume root environment”

Rhizobial bacteria form mutualistic endosymbioses with legumes, in which they induce the formation of root nodules and fix nitrogen. The rhizobia constitute a phylogenetically diverse range of soil bacteria, and it is thought that repeated and independent horizontal gene transfer events led to the spread of symbiotic capabilities to previously non-symbiotic bacteria. Indeed, transferring a plasmid or genomic island containing genes that are important for symbiosis can, in a few cases, turn closely related non-symbiotic bacteria into nitrogen-fixing legume symbionts. However, in most soil bacteria there are additional barriers that prevent symbiotic adaptation following such transfers.

To investigate the nature of these barriers, Marchetti *et al.* sought to drive the experimental evolution of symbiotic behaviour in the root-infecting pathogen *Ralstonia solanacearum*. The authors began by transferring the symbiotic pRalta plasmid from the nitrogen-fixing *Mimosa pudica* symbiont, *Cupriavidus taiwanensis*, into *R. solanacearum* str. GMI1000, creating a new strain, *R. solanacearum* str. CBM124. The presence of pRalta, which carries the genes that are essential for nitrogen-fixing symbiosis, enabled *R. solanacearum* str. CBM124 to stimulate root hair proliferation but not nodulation.

For the experimental evolution of symbiotic traits, the authors repeatedly inoculated *M. pudica* seedlings with *R. solanacearum* str. CBM124 and a gentamicin-resistant derivative (CBM124GenR) and screened for the production of nodules. Three nodules appeared 3–4 weeks after inoculation, and a bacterial clone was isolated from each nodule. The nodulation efficiency of these three isolates was less than that of *C. taiwanensis*, and they were unable to fix nitrogen. Genome resequencing revealed the presence of a single nucleotide polymorphism in each evolved isolate, which introduced a stop codon in either *hrcV* or *hrpG*. HrpG is a master regulator that controls the expression of several virulence determinants, including the HrcV-containing type III secretion system (T3SS) and its associated effectors. Inactivation of *hrcV* and *hrpG* in the parental *R. solanacearum* str. CBM124 recapitulated the nodulation phenotype of the experimentally evolved isolates.

C. taiwanensis invades roots by inducing a root hair to curl around and trap a single bacterium, which can then multiply and trigger the formation of infection threads that allow delivery of the bacteria into the plant cells forming the nodule. *R. solanacearum* str. CBM124 was able to promote root hair proliferation but unable to initiate formation of the infection thread. By contrast, elongated infection threads and nodules could be observed in *M. pudica* inoculated with the *hrcV*-null strain, although the nodules were often irregularly shaped and the bacteria only partially invaded the nodule and remained extracellular. Similarly, the *hrpG*-null strain triggered the formation of infection threads and nodules but, unlike the *hrcV*-null strain, these bacteria were able to intracellularly invade cells in the nodules.

Although the ability to fix nitrogen was not achieved through the experimental evolution process, this study has identified two key adaptive mutations, one that allows nodulation and another that allows intracellular infection. These data also suggest that the barrier to a symbiotic lifestyle, provided by virulence determinants in the recipient, can be overcome by selection for adaptive changes in the legume root environment following the initial transfer of symbiosis genes between phylogenetically diverse bacteria.

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ORIGINAL RESEARCH PAPER Marchetti, M. *et al.* Experimental evolution of a plant pathogen into a legume symbiont. *PLoS Biol.* **8**, e1000280 (2010)