

SELFISH ISLANDS AND CONVERGING PLASMIDS – NEW INSIGHTS INTO EVOLUTION OF ROOT NODULE BACTERIA

Kemanthi Nandasena, Graham O'Hara, Wayne Reeve, Ravi Tiwari and John Howieson
E-mail: kemanthi@murdoch.edu.au

Centre for *Rhizobium* Studies, Murdoch University, Murdoch, 6150, Western Australia.

The coming avalanche of genomic data is bringing opportunities for new insights into the genome architecture and evolution of root nodule bacteria. Comparative genomic analyses of root nodule bacteria reveal that mobile genetic elements ranging from transposons, integrons, genomic islands and plasmids are widespread in these genomes. Genomic islands are plasmid-like DNA regions that are integrated into prokaryotic chromosomes and confer a variety of functions (resistance, degradation, metabolism, pathogenicity, secretion and symbiosis) to the host genome. Acquisition can extend the capacity of the host bacterium to adapt to new environments. Genomic islands that confer nitrogen fixation capacity to nonsymbiotic bacteria are termed 'symbiosis islands' because they carry nodulation and nitrogen fixation genes required for the legume symbiosis as well as genes required for the excision, insertion and transfer of the island.

We have shown the evolution of a diversity of bacteria able to nodulate *Biserrula pelecinus* (a pasture legume species introduced to Australia from the Mediterranean basin) via *in situ* transfer of a symbiosis island from an inoculant strain (Nandasena *et al.*, 2006, 2007). Complete genome sequencing of the inoculant strain and diverse novel strains have revealed that whereas the transferred symbiosis island contains the genes required for nodulation and nitrogen fixation, the diverse bacteria were inferior in their ability to fix nitrogen. We have shown that these "ineffective" strains differentiate into bacteroids and form symbiosomes in young root nodules (10 days after inoculation) but they senesce prematurely. Thus, this genomic island, although termed a symbiosis island based upon the genes it carries, provides no benefit to the legume and therefore a facultative parasitic symbiosis is established instead of the facultative mutualistic association common in legume-rhizobia symbioses. Thus, this island can be considered a selfish island, which spreads between bacteria sharing a common niche (the legume rhizosphere), for its own survival. As a consequence, free-living soil bacteria gain the ability to become a symbiont and explore new niches adding to their evolutionary fitness.

Convergence theory for the evolution of megaplasmids in root nodule bacteria is a second insight we propose based on comparative genomic studies. It appears that strains WSM2304 and WSM1325 of *Rhizobium leguminosarum* bv. *trifolii* gained ancestral forms of two plasmids (pRL32501 and pRL32505) from a common ancestor. The progeny plasmids in WSM2304 converged into a single megaplasmid over evolutionary time whereas the sister plasmids remained separate in WSM1325. *In silico* studies also suggest a similar process for the evolution of megaplasmids in other root nodule bacteria. These discoveries provide insights into the complexities in the evolution of root nodule bacteria and clearly show how lateral transfer of DNA has facilitated the rapid evolution of these organisms in 'quantum leaps'.

Nandasena, K.G., O'Hara, G.W., Tiwari, R.P. and Howieson, J.G. (2006). Symbiotically diverse root-nodule bacteria able to nodulate *Biserrula pelecinus* L. emerge six years after introduction of this legume to Australia. *Applied and Environmental Microbiology*, 72: 7365-7367.

Nandasena, K.G., O'Hara, G.W., Tiwari, R.P. and Howieson, J.G. (2007). *In situ* lateral transfer of symbiosis islands results in rapid evolution of diverse competitive strains of mesorhizobia suboptimal in symbiotic nitrogen fixation on the pasture legume *Biserrula pelecinus* L. *Environmental Microbiology*, 9: 2496-2511.