METAGENOMIC ANALYSIS OF MICROSYMBIONT SELECTION BY THE LEGUME PLANT HOST

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*Rhizobium leguminosarum* bv. *viciae* is able to establish nitrogen-fixing symbioses with legumes of the genera *Pisum, Lens, Lathyrus* and *Vicia*. Classic studies using trap plants (Laguerre et al., Young et al.) provided evidence that different plant hosts are able to select different rhizobial genotypes among those available in a given soil. However, these studies were necessarily limited by the paucity of relevant biodiversity markers. We have now reappraised this problem with the help of genomic tools.

A well-characterized agricultural soil (INRA Bretennieres) was used as source of rhizobia. Plants of *Pisum sativum, Lens culinaris, Vicia sativa* and *V. faba* were used as traps. Isolates from 100 nodules were pooled, and DNA from each pool was sequenced (BGI-Hong Kong; Illumina Hiseq 2000, 500 bp PE libraries, 100 bp reads, 12 Mreads). Reads were quality filtered (FastQC, Trimmomatic), mapped against reference *R. leguminosarum* genomes (Bowtie2, Samtools), and visualized (IGV).

An important fraction of the filtered reads were not recruited by reference genomes, suggesting that plant isolates contain genes that are not present in the reference genomes. For this study, we focused on three conserved genomic regions: 16S-23S rDNA, *atpD* and *nodDABC*, and a Single Nucleotide Polymorphism (SNP) analysis was carried out with multigenomes from each plant. Although the level of polymorphism varied (lowest in the rRNA region), polymorphic sites could be identified that define the specific soil population vs. reference genomes. More importantly, a plant-specific SNP distribution was observed. This could be confirmed with many other regions extracted from the reference genomes (data not shown).

Our results confirm at the genomic level previous observations regarding plant selection of specific genotypes. We expect that further, ongoing comparative studies on differential metagenomic sequences will identify specific gene components of the plant-selected genotypes.

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