

A MLSA-MLST scheme to investigate the real evolutionary dynamics within the *Ralstonia solanacearum* species complex

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The soilborne beta-proteobacterium *Ralstonia solanacearum* is the causing agent of major plant diseases (bacterial wilt on several species, potato brown rot, Moko disease of banana) within tropical and subtropical areas, affecting both cash and subsistence crops. This species complex is constituted of four phylotypes, correlated with the geographical origin of strains (I, Asian; II, American; III, African; IV, Indonesian). Each phylotype is further subdivided in sequevars, on the basis of sequence divergence of the endoglucanase gene (*egl*); some of these sequevars have been associated to specific ecological features (host range, virulence at cool temperatures...). Genomic structure and phylogeny of this species complex is now well understood, thank to recent CGH studies (2) and sequencing of several complete genomes. The subdivision in four phylotypes was clearly demonstrated, but the existence of different clades within each phylotype still had to be validated. The high genotypic and phenotypic plasticity of this organism has been illustrated by emergence of new pathogenic variants (3). It was demonstrated that this bacterium, naturally competent, is potentially subjected to recombination and horizontal genetic transfer (HGT). However, the real degrees of recombination occurring in natural populations, the dominant reproductive mode of this bacterium, the selection pressures structuring these populations, are still largely unknown. To clarify both phylogeny and evolutionary dynamics of the *R. solanacearum* species complex, we developed a MLSA-MLST scheme on a collection of 88 *R. solanacearum* strains classified in the four phylotypes and 51 sequevars described to date, and one strain of each of the close species *R. syzygii*, *R. pickettii*, *R. mannitolylitica* and *R. insidiosa*. Genes were chosen following previous MLSA approach (1) and reference studies; all were (i) evenly distributed across the two replicons, and distant of at least 100 kb, (ii) present in one single copy in the genomes. They consisted in six housekeeping genes (*ppsA*, *rpIB*, *gdhA*, *leuS*, *adk*, *gyrB*), the DNA mismatch repair gene *mutS*, and two virulence-associated genes (*egl*, *fliC*).

Phylogenies reconstructed from individual genes, and with concatenated genes, were globally congruent with each other. They allowed identifying several phylogenetically differentiated subdivisions, named clades, within some of the phylotypes. Within the phylotype I, one single clade was found. Within the phylotype II, the subclusters IIA and IIB were validated and four clades were found: (i) "brown rot" IIB/sequevar 1, 2 and Moko sequevar 3; (ii) sequevar 4 (Moko and emergent strains); (iii) "South-Eastern USA biovar 1" strains, or sequevar 7; (iv) "Antillean biovar 1" strains, and Moko sequevar 6 strains. Within the phylotype III, two clades were identified: (i) Austral Africa and Indian Ocean strains, and (ii) Central and Western African strains. Within the phylotype IV, two clades were found: (i) blood disease bacterium and Indonesian *R. solanacearum* strains, (ii) *R. syzygii*.

The population structure of *R. solanacearum* was assessed by the MLST approach, at the global scale and at the phylotype scale. The global population structure appeared to be clonal; however phylotypes clearly differed in structure: phylotypes I and III displayed a recombining population structure, whereas phylotype II was highly clonal.

Analyses of the different evolutionary forces structuring the *R. solanacearum* species complex are ongoing, and will be presented and discussed. A collection of reference strains for each clade will be proposed.

References:

1. Castillo, J. A., and J. T. Greenberg. 2007. *Appl. Environ. Microbiol.* 73: 1225-1238.
2. Guidot *et al.*, 2007. *J. Bacteriol.* 189: 377-387.
3. Wicker *et al.*, 2007. *Appl. Environ. Microbiol.* 73: 6790-6801.