

Year of CfP: 2008

Project No 0802-012 completed

Project title: Analysis of <i>Xanthomonas albilineans</i> Gene Expression during Sugarcane Leaf Scald Pathogenesis

Unit managing the project: BGPI (Biology and Genetics of plant/pathogen interactions) (CIRAD, INRA Montpellier SupAgro)

Project leader: Monique Royer (monique.royer(a)cirad.fr)

Country involved in the project: USA

Research units from the Foundation's scientific network involved: LGDP

Sub-thematic axis: IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*)

Objectives:

The xylem-invading mechanisms of plant pathogenic bacteria are still not well known. *Xanthomonas albilineans* is the causal agent of leaf scald disease of sugarcane, one of the major diseases of sugarcane, and it is one of the most ancient species of the *Xanthomonas* genus. This pathogen multiplies in the xylem and systemically colonizes the entire host plant. *X. albilineans* has several unusual characteristics that distinguish its pathogenicity from other *xanthomonas*.

The recent sequencing and annotation of the genome of *X. albilineans* revealed that the size of this genome (3.7 Mb) was reduced in comparison to the sizes of the genomes of other *xanthomonas* sequenced to date (approximately 5 Mb). Additionally, *X. albilineans* possesses 518 genes that are not conserved in other *xanthomonas*, but is missing the Hrp type III secretion system (T3SS) which is found or thought to be present in most other pathogenic *xanthomonas*. The Hrp system is used for injection of protein pathogenicity effectors into plant cells.

It is indispensable to identify new candidate genes potentially involved in pathogenesis. The objective of this project is therefore to investigate, using microarray technology, the full breadth of the response of *X. albilineans* to sugarcane host environment during the colonization of the xylem and also during the epiphytic life of the pathogen.

Bacterial gene expression will be quantified using the microarray technology that was recently successfully used by Professor Caitilyn Allen to investigate the full breadth of the response of *Ralstonia solanacearum* to tomato environment.

Action carried-out and results obtained:

Action 1: Carrying out experiments to study gene expression during sugarcane leaf scald pathogenesis

Xanthomonas albilineans is the causal agent of leaf scald disease of sugarcane, and a xylem-invading plant pathogenic bacterium. The objective of this project is to identify candidate genes by investigating the full breadth of the response of *X. albilineans* to sugarcane host environment during the colonization of the xylem.

Bacterial gene expression will be quantified using the microarray technology that was recently successfully used by Professor Caitilyn Allen to investigate the full breadth of the response of *Ralstonia solanacearum* to tomato environment.

Custom microarrays specific to the transcriptome of the *X. albilineans* strain GPE PC73 were designed and produced by NimbleGen (Madison, WI, USA). These arrays are specific to following sequences:

- the 3209 protein-coding sequences annotated in the genome of the *X. albilineans* strain GPE PC73,
- all intergenic regions identified in the genome of the *X. albilineans* strain GPE PC73,
- 505 ESTs of *Saccharum officinarum* corresponding to either orthologs of rice genes involved in defense mechanisms or either EST present in many copies (30 à 300) in the databases which therefore may be constitutively expressed.

We prepared sugarcane plants infected by the *X. albilineans* strain GPE PC73. We prepared total RNA from several samples collected on the stem of these sugarcane infected plants. To prepare these total RNA, we used the protocol developed by Professor C. Allen to prepare RNA from samples collected on the stem of tomato plants infected by *R. solanacearum*, a pathogenic bacterium that spreads into the xylem. We also prepared total RNA from liquid cultures of the *X. albilineans* strain GPE PC73. These RNA will be soon analysed by Nimblegen using the arrays produced as part of this project. The Professor Caitilyn Allen's team will participate to the statistical analyses of the microarray experiments. Differential analyses of microarrays experiments of RNA prepared from samples collected on the stem of infected sugarcane plants or from liquid cultures will lead to the identification of gene expressed during sugarcane leaf scald pathogenesis.

Action 2: Teaching Activities at Montpellier SupAgro

- Diagnostique et gestions des maladies bactériennes des plantes tropicales (Diagnosis and management of bacterial diseases of tropical crops). Three-hour intensive module for 22 Tropical IPM Masters students from francophone Africa at Montpellier SupAgro (January 26, 2009).
- Biologie évolutive et diversité du vivant (Evolutionary Biology and Diversity of Life) – Two-hour lecture on the evolution and phylogeny of plant pathogenic bacteria. Fifteen Montpellier SupAgro Master's students (April 20, 2009).
- Member of the Advisory Committee of Ph.D. student Melanie Marguerettaz (ED SIBAGHE, Université Montpellier 2, France; Monique Royer, Thesis Advisor)

Prospects for the future:

Action 1: The candidate genes identified by the microarray analyses will be further studied. The expression of these genes will be studied by quantitative RT-PCR in the samples collected from the stem of sugarcane plants inoculated with the *X. albilineans* strain GPE PC73. Functional analysis of these genes will be performed with knockout mutants in which these genes will be inactivated. Professor Caitilyn Allen will participate to this future project.

Action 2: Professor Caitilyn Allen will participate to the future student exchange programs between University of Wisconsin-Madison and Montpellier SupAgro. She also will participate to the Advisory Committees of the future Ph.D. students of BGPI who will study pathogenicity of *X. albilineans*.

Total Agropolis Fondation funding: € 21,360 (travel, housing and direct costs link to the conduct of experiments)

Funding categorie(s): Agropolis Fondation visiting fellowship (senior scientist, less than 12 months)

Project duration: 15 October 2008 - 30 June 2010

Keywords: *Xanthomonas albilineans*, pathogenicity, gene expression, microarrays