Year of CfP: 2007

Project No: 07012 Completed

Project title: Unravelling pathogenecity of *Xanthomonas albilineans*, an original model in plant pathology

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

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

Country involved in the project: USA

Subthematic axes: IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*)

Objectives:

The xylem of plants is invaded by numerous bacterial plant pathogens and symbionts. However, no or very few data are available to date regarding the mechanisms that confer the capacity of these pathogens to colonize the xylem of their host. The bacterium *Xanthomonas albilineans* is a systemic, xylem-invading pathogen that causes leaf scald disease of sugarcane. The symptoms of the disease vary from a single white line on the leaves to the death of the plant, via bleaching and necrosis of the leaves.

X. albilineans is a problem in over 60 tropical and subtropical countries which produce sugarcane. This bacterium produces a unique and specific toxin, called albicidin that causes leaf scald symptoms. Albicidin is a potent DNA gyrase inhibitor that inhibits the replication of proplastic DNA and consequently blocks the differentiation of chloroplasts. The albicidin biosynthesis genes were cloned and sequenced at UMR BGPI in collaboration with Professor Dean W. Gabriel from the University of Florida. Although albicidin genes are essential in pathogenicity of *X. albilineans*, other genes, so far unknown, also play an important role in host-pathogen interactions.

To facilitate the identification of these unknown pathogenicity genes, the entire genome of *X. albilineans* was recently sequenced at Genoscope (Evry, France). The analysis of this genome confirmed that *X. albilineans* is an original model system in the world of plant pathology. The size of this genome (3.7 Mb) is smaller than those of other *Xanthomonas* species (~ 5 Mb). This genome does not possess the type three secretion system that is required in plant pathogenic bacteria for injection of protein pathogenicity effectors into plant cells. Additionally, this genome possesses 754 open readin frames that have not been found in any other *Xanthomonas* species. The objective of UMR BGPI is to identify *X. albilineans* pathogenicity genes, giving rise to prospects for the design of new control methods of plant diseases.

The objectives of the present project are:

- to consolidate the existing collaboration between Cirad and the University of Florida by supporting a three months visit of a technician of Cirad who will learn different molecular methods and tools recently developed there
- to structure a new collaboration with the group of Professor Caitilyn Allen from the University of Wisconsin–Madison that has the scientific knowledge to study colonization of plants by pathogenic bacteria. In this context, Dr Monique Royer from Cirad in Montpellier and Dr Philippe Rott from University of Florida will go for one week to the University of Wisconsin-Madison.

Action carried-out and results obtained:

Action 1: Isabelle Pieretti spent three months at the University of Florida from 1st February to 30th April 2008 under the supervision of Prof. Dean W. Gabriel. Her studies allowed us to identify genes that are specific to a highly pathogenic strain of *X. albilineans* using the Suppression subtractive hybridization technique. This training in the USA also allowed I. Pieretti to improve her oral and written English language skills.

Result of Action 1: Suppression subtractive hybridization (SSH) is a differential screening method coupled to polymerase chain reaction (PCR) that was developed to compare bacterial genomes. It can be useful to identify unique or strain-specific DNA fragments when comparing two bacterial genomes that are relatively close. We applied SSH to two strains of *Xanthomonas albilineans* from Florida (XaFL07-1 and Xa23R1) that belong to different genetic and pathogenicity groups, in order to identify genes putatively involved in leaf surface and vascular colonization of sugarcane plants. Highly pathogenic strain XaFL07-1 was used as the tester strain. This strain belongs to the same genetic group as strain GPE PC73 of *X. albilineans* from Guadeloupe whose genome was recently sequenced and annotated. Strain Xa23R1 is less pathogenic than strain XaFL07-1 and is not spread aerially. It was used as the driver (subtractive) strain, i.e. the control DNA against which the tester strain DNA was compared. A library of 576 clones enriched for XaFL07-1 DNA was generated with the PCR-select Bacterial Genome Subtraction Kit (Clontech). After verification by Southern blot hybridization, 188 clones were found specific for strain XaFL07-1. These 188 clones were sequenced and the sequences were then blasted against the complete genome of *X. albilineans* strain GPE PC73. Comparative analyses of these sequences resulted in the elimination of 83 XaFL07-1 sequences because they were absent in the genome of pathologically similar strain GPE PC73, or because they were plasmid, phage or transposase sequences. The remaining 105 sequences were chosen to be validated by PCR and for further analysis, especially functional genetics in strain GPE PC73. These sequences include genes encoding for hemagglutinins and hemolysins, a TonB-dependant outer membrane receptor, a large membrane receptor, a DNA methyltransferase, and several permeases or transporters.

Action 2: Monique Royer and Philippe Rott visited the group of Prof. Caitilyn Allen at University of Wisconsin-Madison from 15th to 18th of April 2008. During her stay in the USA, Monique Royer also visited the group of Prof. Dean W. Gabriel at University of Florida.

Result of Action 2: M. Royer and P. Rott discussed with C. Allen the work that was performed on *X. albilineans* at UMR BGPI in Montpellier (M. Royer) and at University of Florida in Gainesville (P. Rott), in order to develop a new collaboration. It was agreed that Prof. C. Allen will spend a sabbatical of six months in Montpellier, from January to June 2009. During this period, Prof. C. Allen will teach classes in plant pathology at Montpellier SupAgro and will perform research studies on *X. albilineans* at UMR BGPI. Prof. C. Allen will especially try to identify genes induced in *X. albilineans* during colonization of sugarcane by this pathogen, using DNA microarrays. This project was submitted to and accepted by RTRA in the frame of RTRA's APP 2008. M. Royer and P. Rott gave a seminar to the plant pathology department of University of Wisconsin that was entitled: "Genomics and functional genomics of *Xanthomonas albilineans*, an unusual xanthomonad that causes leaf scald disease of sugarcane". M. Royer and P. Rott also met researchers of the departments of pathology and microbiology of the University of Wisconsin-Madison who specialize in the biosynthesis of antibiotics and the finding of new molecules produced by various microorganisms.

Prospects for the future:

The work performed by Isabelle Pieretti allowed us to identify several genes putatively involved in pathogenicity of *X. albilineans*. Professeur Caitilyn Allen will be hosted by UMR BGPI from January to June 2009 in the frame of a new RTRA project (N° 0802-012). This new project will allow us to identify additional pathogenicity candidate genes. All these new candidate genes will be further investigated, especially by functional analyses of *X. albilineans* strain GPE PC73. These studies will be performed in the frame of collaborations with University of Florida and University of Wisconsin-Madison.

Total Agropolis Fondation funding: € 12,749 (travel expenses)

Funding categorie(s): Agropolis Fondation small grants (overseas travel grants for doctoral and post-doctoral scientists, development of a project)

Project duration: 2 January 2008 – 30 June 2008

Keywords: *Xanthomonas albilineans* – pathogenicity – plant – toxins – sugarcane – leaf scald disease