

## Ad hoc support : Génétique Bactérie

### Research programme on the genetic processes that allow the establishment of bacterial pathogens in their hosts

#### ABSTRACT

Project 1: Ax21 from *Xanthomonas* - its role in quorum sensing and as an elicitor of plant defense. The Gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae* causes bacterial leaf blight of rice, a devastating bacterial disease affecting rice plants in most rice-cultivating areas. Genetic resistances proved as being most efficient in controlling the disease, among them the resistance gene *Xa21* (Song et al. 1995, *Science* 270: 1804-1806). Previous work had indicated that Ax21, a short peptide derived from a secreted protein of *Xanthomonas*, might trigger a defense response in rice upon perception by the receptor-like kinase *Xa21*, ultimately leading to plant resistance (Lee et al. 2009, *Science* 326: 850-853). Since this view was recently challenged, experiments were performed to elucidate the role of Ax21 as a trigger of plant defense. Wild-type bacteria and mutants in *ax21* and *raxST* were inoculated into *Xa21*-transgenic rice plants (TP309-*XA21*) and symptoms were recorded. Surprisingly, the *ax21* mutant of *X. oryzae* pv. *oryzae* could not overcome *Xa21*-mediated resistance, while the *raxST* mutant (*rax* = required for *AvrXa21* activity) could overcome resistance, as reported by da Silva et al. in 2004 (*Mol. Plant Microbe Interact.* 17: 593-601). It is possible that Ax21 is only one of two or more ligands that are involved in *Xa21*-mediated resistance. In order to gain new insight into the pathogenicity of *X. oryzae* pv. *oryzae*, several strains were selected and sent for genome sequencing using Illumina technology.

Project 2: Banana *Xanthomonas* Wilt - an emergent pathogen in East Africa

Banana *Xanthomonas* Wilt (BXW), caused by pathogenic strains of *Xanthomonas vasicola* pv. *musacearum* (syn. *X. campestris* pv. *musacearum*), is a new epidemic disease in several East African countries, incl. Uganda, Kenya, Congo, Rwanda and Burundi (Tripathi et al. 2009, *Plant Disease* 93: 440-451). Transgenic *Xa21* banana plants have been reported to provide protection from *Xanthomonas* infection (Leena Tripathi, pers. commun.). We therefore decided to study the BXW pathogen. For comparative genomics, three strains isolated from enset plants and originating from Ethiopia, the probable source of inoculum of the current outbreak in East and Central Africa, have been sent for sequencing using Illumina technology. We established a protocol to genetically modify *X. vasicola* pv. *musacearum* by DNA transformation (via electroporation). This technique will allow us to study the *Xanthomonas*-banana pathosystem in greater detail. The IRD laboratory had previously identified a candidate peptide, RapX, that could be secreted from xanthomonads via the type I secretion system (the *Rax* system) which is also involved in triggering *Xa21*-mediated resistance in rice (Guillaume Robin 2010, PhD thesis, University Montpellier 2, France). It was of interest to see whether or not this peptide is present in *X. vasicola* pv. *musacearum*. 12 strains of *X. vasicola* pv. *musacearum* originating from Ethiopia, Uganda, Congo, Rwanda and Tanzania from the French collection of bacterial plant pathogens (<http://www-intranet.angers.inra.fr/cfbp/>) were chosen for study. Upon PCR amplification using primers annealing to *raxST* and *raxA*, DNA amplicons were sequenced. All 12 strains encoded the same double glycine leader peptide, MSRHVAWQRTAARRRHRYAGGQHGRASAAAGG, the mature part of which (QHGRASAAAGG) is identical to that from castor oil plant pathogens and closely related to that from xanthomonads infecting cassava, mango and beans, among others. We were also interested to see whether or not *X. vasicola* pv. *musacearum* encodes TAL (Transcription Activator Like) type III effectors, a major virulence factor of xanthomonads infecting rice and Citrus. Using a hybridization probe derived from *X. oryzae* pv. *oryzae*, Southern blot analyses did not show any signals for TAL effector genes in the 12 strains of *X. vasicola* pv. *musacearum*. Also PCR using TAL effector-specific primers from *X. oryzae* pv. *oryzae* did not provide evidence for the existence of TAL effectors in *X. vasicola* pv. *musacearum*. While still not decisive since TAL effector genes from *X. vasicola* pv. *musacearum* could have sequence polymorphisms to escape detection via Southern blots and PCR, our findings are nevertheless corroborated by draft genome sequences which do not include candidate TAL effector genes in their assemblies.

**Keywords :** Plant, Rice, Interaction, Gene expression, Plant disease

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**Project leader :** Michel Nicole

**Project leader's institution :** IRD

**Project leader's RU :** IPME-PHIM

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## PERSPECTIVES

Future studies will elucidate the role of Ax21 and RapX in quorum sensing, another phenomenon that is controlled by the Rax system. The ability to genetically modify *X. vasicola* pv. *musacearum* will help to decipher the *Xanthomonas*-banana pathosystem. Our new genomic resources will be instrumental to gain deeper insight into the role of the Rax system and other pathogenicity factors to provoke plant diseases. This initiative, which was generously supported by the Agropolis Foundation, lays the foundation stone for a future project on BXW within the Roots, Tubers, and Banana consortium (<http://www.rtb.cgiar.org/>) under the umbrella of the Consultative Group on International Agricultural Research (CGIAR, <http://www.cgiar.org/>).