SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA16107
STSM title: "Characterization of the mode of action of a short TAL effector from *Xanthomonas citri*"
STSM start and end date: 28/01/2019 to 10/03/2019
Grantee name: Dr. Roeschlin Roxana

PURPOSE OF THE STSM:

Unpublished data obtained during my PhD and postdoc indicate that a natural variant of *Xanthomonas*, named *X. citri* AT, triggers a host-specific defense response in *Citrus limon*, *C. sinensis*, and *Nicotiana benthamiana*. Moreover, we have identified that the bacterial protein causing the deployment of the hypersensitive response (HR) is a short TAL effector (7.5 repeats) variant, termed PthA4_AT, of the PthA4 TAL effector (TALE), which is an important virulence factor for the pathogen. The mode of action of PthA4_AT is via the plant nucleus and that the effector can bind DNA in vitro in a code-dependent manner and activate transcription of at least one target gene. However, due to the low number of repeats, the target DNA sequence is only 9 bp long and it is present many times within the plant genome. The large number of putative targets indicate that a more detailed understanding of the mode of action of PthA4_AT is needed to successfully identify the cause for the induction of HR.
The main objective of this STSM was the characterization of the mode of action of PthA4_AT by evaluating the specificity of this TALE to trigger gene expression to initiate the search of target(s) gene(s) responsible for the HR response.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

During the STSM, different approches were performed to decipher the mode of action of PthA4_AT and elucidate the target(s) gene(s) resposible for the triggering of HR in *N. benthamiana* plants.
Using the modular TALE construction kit available at the Dr. Boch's laboratory in Hannover, I had built an artificial PthA4_AT (a-PthA4_AT) with the same RVDs as PthA4_AT. With this a-PthA4_AT, I had evaluated the timing of HR development after agroinfiltration in *N. benthamiana* and assessed how efficient are the a-PthA4AT and PthA4AT to induce expression of *GUS* reporter genes though the binding to putative target binding sequences. Moreover, in order to assess PthA4_AT specificity and narrow the number of target genes, I had cloned different artifitial TALE modules by changing different RVDs: some with more specific RVDs at positions N* and NS, some with different RVDs in individual positions to re-target to other sites and others with extra RVDs at the C-terminal end of the repeat array (9.5 repeats). All these contructions were evaluated for HR development in *N. benthamiana* and compared with a-pthA4AT and pthA4-AT. Finally, during the STSM, I had evaluated if those modules that induce HR were able to induce *GUS* expression on different putative promoter targets.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

By combining the information obtained from the experiments performed during the STSM, we could limit the number of putative targets of PthA4_AT. First of all, we have demonstrated that the a-PthA4_AT induced HR at the same level as PthA4_AT in *N. benthamiana*. Moreover, we could elucidate that the RVD "N*" at position 2 in PthA4_AT is binding uniquely to the nucleotide T, and only triggers HR if the effector binding site contains the nucleotide T. However, we could not find a unique binding code or HR trigerring specificity for the RVD "NS" at position 4. With our results, we found that "NS" does not trigger HR if it meets A in the fourth position of the EBE, but still does so if it binds G, C or T. Finally, the extension of the repeat array to 9.5 repeats with different RVDs at the C-terminal end demonstrated that some of them are still able to induce HR, suggesting that more that one resistance gene could be responsible of HR.
In regard of these results, at the end of the STSM we decided to construct longer TALEs with 10.5 repeats, which will be done in Spain during the next weeks.

FUTURE COLLABORATIONS (if applicable)

Promising results were obtained during the STSM and we could start to narrow down the list of possible targets of PthA4_AT.
Moreover, during the STSM all TALEs were subcloned in *Xanthomonas* expression vectors to assay if they could trigger HR in other plants. We proposed a new colaboration project in which the different constructs will be transformed in *X. oryzae* to assay HR in rice at the Dr. Boch's laboratory in Germany and in *X. citri* to assay HR in citrus at the Dr. Marano's laboratory in Argentina in the following months. Moreover, the new, longer TALE contructs obtained at the end of the STSM will be evaluated in *N. benthamiana*, and RNAseq experiments to narrow down the putative HR-inducer target genes will be performed at Dr. Gadea's laboratory in Spain. This knowledge will help to rationally exploit the plant immune system as a biotechnological approach to manage different diseases, and reveal biological functionality for short TAL effectors.