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: Functional study of the Asian *Xanthomonas oryzae* pv. *oryzae* CRISPR/*cas* system and evaluation of OP-like bacteriophages on different clades of *X. oryzae*
STSM start and end date: 20/01/2019 to 16/02/2019
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PURPOSE OF THE STSM

CRISPR/*cas* systems, a defence system against alien DNA, have been identified in several bacteria and in most archaea. However, it is not clear if these systems are always functional, i.e. whether they can acquire new spacers and whether they can protect bacteria against subsequent attack by related bacteriophages. In the genome of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), e.g. strain PXO99^{A}, a CRISPR locus is present and several CRISPR-associated proteins are encoded in the genome. Based on these observations we supposed that the CRISPR system in *Xoo* strains originating from Asia might be functional. This functional study was managed using PCR amplification before and after bacteriophage challenge to identify new spacers in the genomes of *Xoo*.
Recently, Enviroinvest Corp. isolated several bacteriophages against *Xoo* in the Philippines and in Vietnam. All of them belong to the OP$_2$-like group. The second purpose of the STSM was to evaluate whether these bacteriophages can also infect African strains of *Xoo* and/or other pathovars of the species *X. oryzae*.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

Functional study of the Asian *Xoo* CRISPR/*cas* system
I grew three Asian *Xoo* strains representative of different genetic lineages (PXO99^{A}, VXO16 and A3842) and I amplified (using colony PCR with boiled cells) the leader-proximal DNA fragment of the CRISPR/*cas* locus with specific primer pairs: one primer matching to the *cas* gene next to the leader sequence (serving as a universal primer for all strains) and the other primer matching the most recently acquired spacer (serving as a specific primer for each strain). These PCR products served as control in the following experiments.
I tried to identify acquisition of new spacer(s) in the genomes of *Xoo* upon bacteriophage challenge. I infected all three *Xoo* strains with bacteriophages representative of the two OP$_2$-like lineages (both from Vietnam). Upon 24 hours of co-incubation at 28 °C, I isolated surviving

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bacteria by plating on agar plates. Boiled cells of the survivor bacteria served as template for PCR amplification and DNA amplicons were analysed by 1.2% agarose gel electrophoresis. Any changes in the genome would be visible on the gel (indicative of the appearance of a new spacer).

Evaluation of OP_2-like bacteriophages on different clades of *X. oryzae*

I tested the newly isolated bacteriophages from Enviroinvest on representative strains of *Xoo* and *Xoc* from Asia and Africa, on strains of *X. oryzae* from the USA and on *X. oryzae* isolated from southern cutgrass (*Leersia hexandra*). Altogether I infected 17 different *X. oryzae* strains with two bacteriophages from Philippines and with two bacteriophages from Vietnam. I prepared 10fold dilution series of the bacteriophage lysates and efficiencies of plating were determined with the bacterial strains using classic drop-on-lawn technique on soft agar plates. After 18-24 hours I analyzed the plaques and determinad the host-specificity of each examined bacteriophage.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Functional study of the Asian *Xoo* CRISPR/*cas* system

I tried to identify new spacer(s) in the genomes of *Xoo* upon bacteriophage challenge. I got several survivor colonies from each mixture. In total, I harvested 6 x 6 colonies from these mixtures (challenge of three Xoo strains with two bacteriophages) and I compared the size of the PCR products before and after the bacteriophage treatment by agarose gel electrophoresis. I did not observe any changes in the size of the PCR products. This analysis indicates that the surviving bacteria had not acquired new spacers upon bacteriophage infection. These colonies were tested again with bacteriophages and turned out to be completely resistant. It is likely that these evamined survivor colonies are receptor mutants. These receptor mutants will be very useful when characterising new bacteriophage isolates.

Evaluation of OP_2-like bacteriophages on different clades of *X. oryzae*

I tested the newly isolated bacteriophages from Enviroinvest on representative strains of *X. oryzae*. Altogether I infected 17 different *X. oryzae* strains (14 strains from the IRD collection and three strains from the Enviroinvest collection) with two bacteriophages from Philippines and with two bacteriophages from Vietnam. When I analysed the plaques I observed that the new bacteriophages from Vietnam and from the Phillipines were completely unefficient against the strains from IRD collection. Only the OP_2 bacteriophage could lyse a few *Xoo* strains (VXO16 from Vietnam and A3842 from India). I found two single plaques on the plates with *Xoo* strains from Pécs that were caused the XPV1 bacteriophage from Vietnam. I isolated phages from these plaques and propagated them on plates with *Xoo* strains from Hungary by adding 5 ml of liquid medium on top of the soft agar containing 100 μl of a bacterial suspension at OD_{600}=0.6. After two days at room temperature I harvested the supernatant and purified the bacteriophages y sterile filtration. After that I infected some bacterial strains with these newly isolated bacteriophages using routine methods. These new bacteriophages infected some *Xoo* strains ($PXO99^A$ from Philippines, VXO16 from Vietnam and A3842 from India) with very good efficiency. These three strains were completely resistenst against the original XPV1. I denominated these newly isolated strains XPV1/h1 and XPV1/h2.

FUTURE COLLABORATIONS (if applicable)

In order to identify new spacers in the *Xoo* genome, we would like to try other method(s) to infect the bacterium with bacteriophages (we could use semi-solid medium instead of liquid medium for the treatment).
IRD possesses numerous *Xoo* and *Xoc* strains in its collection originating from several countries. It would therefore be interesting to test these newly isolated bacteriophages (XPV1/h1 and XPV1/h2) on other strains of *X. oryzae*. We would like to characterise these two new phages. Morphology of phages will be revealed by transmission election microscopy. Lytic activity of phages will be assayed spectrophotometrically by monitoring the changes in the optical densitiy. We would like to sequence the whole genome of these phages and compare them with the original XPV1 phage using bioinformatic tools.
It is planned to apply for an EuroStars grant using *Xanthomonas*-specific bacteriophages as a biocontrol agent.