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: Genetic characterization of *Xylella fastidiosa* lipidic pathways
STSM start and end date: 10/02/2019 to 24/03/2019
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PURPOSE OF THE STSM:

In our previous research it was demonstrated that, within a growing time frame (7 to 11 days), lipid composition of *Xylella fastidiosa* cells and its medium filtrate changed significantly. In particular, the culture filtrate showed an increase in free unsaturated fatty acids and oxylipins. Among unsaturated fatty acids there are the Diffusible Signalling Factors, involved in quorum sensing cell-cell cross talk. Oxylipins themselves could have a role in cell signalling upstream biofilm formation, as previously demonstrated for *P. aeruginosa*. Interestingly, among the oxylipins found as increased in *X. fastidiosa* culture medium, two of them, 10HOME and 10HPOME, are involved in *P. aeruginosa* biofilm formation. In addition, in our unpublished work, 10HPOME was found increased in *X. fastidiosa*-infected olive samples.
Starting from these results, we aimed to characterize the role for these oxylipins in *X. fastidiosa*. Specifically, the aim was to create deletion mutants for oleate lipoxygenases and hydroperoxide diole synthases, in order to assess the impact of such enzymatic activities on bacterial lifestyle. The goal was to evaluate if oxylipins production varies significantly and if the effect of oxylipin metabolic pathways has an impact on the bacterial capability of forming biofilm, as demonstrated in *P. aeruginosa*.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

In *P. aeruginosa* PA2077 and PA2078 genes were previously characterized (Estupiñàn et al., 2014) as, respectively, an oleate lipoxygenase and a hydroperoxide diole synthases. In silico search was performed and allowed to find sequences in *X. fastidiosa*, both in strain *X. fastidiosa* subsp. *fastidiosa* Temecula1 and *X. fastidiosa* subsp. *pauca* De Donno, harboring the same conserved functional domains. The locus tag for the putative oleate lipoxygenase is PD1708 in the Temecula1 genome and B9J09_01555 in the De Donno genome. The locus tag for the putative hydroperoxide diole synthases is PD0744 in the Temecula1 genome and B9J09_07200 in the De Donno genome. The objective of the mission was the creation of *X. fastidiosa* mutants for both genes, in each strain. For both strains primers suitable for PCR and subsequent fusion PCR were designed. The rationale of fusion PCR is to amplify a construct made by the upstream and downstream flanking regions of the gene of interest for site-specific mutagenesis, using an antibiotic-resistance cassette as a marker. This way the construct can recombine at the target locus, thus replacing the gene of interest with the marker cassette. Even if Olive Quick Decline Syndrome in Italy is caused by De Donno strain, and all my previous analyses were done with the De Donno strain, for the deletion experiments Temecula1 strain was chosen, in addition to De Donno. Reasons for choosing Temecula1 are the following: the high similarity of the chosen sequences, making it a suitable model, and the relative easiness of transformation with respect to De Donno. Temecula1, in fact, can incorporate exogenous DNA through natural competence, while De Donno strain cells require being electroporated to acquire exogenous DNA. Both strains will be engineered. So far, in addition to the in silico work, I collected

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stocks of both strains, I extracted DNA from Temecula1 and performed PCRs and fusion PCRs to obtain the constructs required for transformation. Regarding the kanamycin resistance cassette, I amplified and purified it from DNA extracted from an *E. coli* strain harboring the pUK4C plasmid. In this period I also got familiar with greenhouse work and with the basics of microfluidic chamber techniques, that will be useful for further assays that I will perform in the next months after the STSM.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Stocks of both strains were collected after culturing in PW media and stored in glycerol at -80 °C. PD3 media and PD3-kanamycin media for next steps were prepared and stored at 4 °C. Temecula1 and De Donno cells for DNA extraction were collected and stored at -20 °C in water. Temecula1 DNA was extracted and quantified. Kanamycin cassette was extracted from a recombinant *E. coli* strain harboring the pUK4C plasmid and then purified. PCR of *X. fastidiosa* DNA and kanamycin-resistance cassette purified DNA were set up and performed in order to obtain amplicons of: upstream and downstream regions of PD1708 gene; upstream and downstream region of PD0744 gene, kanamycin resistance cassette. Amplicons were purified and the purified products were amplified by fusion PCR, to obtain two fusion products, one for each gene of interest. PCR and fusion PCR reactions were assessed through gel electrophoresis, which proved the presence of the desired amplicons (See Figs.1 and 2). These constructs were purified from the gel and stored at -20 °C: they will be used to transform Temecula1 cells, currently growing, by natural competence. Generally, with the STSM I got familiar, after mass spectrometry oriented background, with microbiological and molecular biology techniques, with a special mention for the fusion PCR. I also acquired the basics of microfluidic chamber technique.

Fig.1 Amplicons of: upstream (A) and downstream construct (B) for PD1708 gene; upstream (C) and downstream (D) construct for PD0744 gene; kanamycin-cassette (E); 1kb ladder (F)

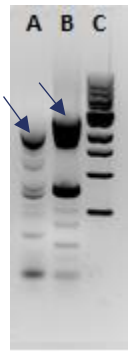

Fig.2 Amplicons of: PD1708 fusion product (A, arrow) PD0744 fusion product (B, arrow) 1kb ladder (C)

FUTURE COLLABORATIONS (if applicable)

The collaboration will continue in the next months to complete deletion mutagenesis experiments and to perform *in vitro* and *in planta* assays with the mutant Temecula 1 strains. At the same time, I will try to transform De Donno strain with electroporation and to perform the same *in vitro* and *in planta* assays. Hopefully, deletion mutants of each type will be studied in a xylem-like environment using a micro-fluidic chamber. We expect that my research here will produce most of the data for a future joint publication with the host lab.