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: "Unravelling the antibiofilm effects of plant-derived compounds at non-lethal concentrations against the phytopathogenic bacteria *Xylella fastidiosa*"
STSM start and end date: 08/01/2018 to 08/03/2018
Grantee name: Luca De Vincenti

PURPOSE OF THE STSM

The recent outbreaks of *Xylella fastidiosa* in Europe has prompted me to deepen my understaning on this phytopathogen bacteria, especially in the mechanisms involved in biofilm formation, because it plays an important role in insect and plant colonization. The purpose of my STSM is to develop innovative and eco-friendly strategies to counteract *X. fastidiosa* biofilm. The use of sub-lethal doses of bio-inspired molecules and nanoparticles offer an elegant way to interfere with specific key-steps that orchestrate biofilm formation, increasing cell susceptibility to lower concentrations of biocides and extending the efficacy of the current arsenal of antimicrobial agents.
During these months in the laboratory of Prof. De La Fuente at Auburn Univeristy I had the opportunity to increase my experience in manipulating several strains of *X. fastidiosa*. I acquired and developed new methodologies and experimental techniques, enriching and improving my research skills. For the first time I performed experiments in planta under greenhouse conditions, using tobacco as model plant. Once infected, I will compare the evolution of the plant fitness in presence and in absence of my compounds, from the appearance of the first symptoms until severe desiccation. Lastly, I enhanced some other personal skills such as team-working and communication skills.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

During my STSM at Auburn Univerisity I performed in vitro and in planta assays using two strains of *Xylella fastidiosa* belonging to two different subspecies: *fastidiosa* (WM1-1 strain, isolated from grape) and *multiplex* (BB08-1 strain, isolated from blueberry). For the in vitro experiments I evaluated the antibiofilm ability of sub-lethal concentration of different compounds: salicylic acid, n-acetylcisteine (from 12 to 0.001 mM) and zinc oxide nanoparticles (from 100 to 10 ppm). First, I determined the ability of each compound to inhibit planktonic growth, an undesirable effect for a potential anti-biofilm molecule that must, on the contrary, acts at sub-lethal doses, disarming microorganisms without killing them. Briefly, one week old colonies grown on Periwinkle wilt (PW) agar plates were restreaked onto new PW plates and cultured at 28°C for another week before use. After that, PD3 liquid medium was used to resuspend the cells and growth curves were generated using a microplate reader by measuring the optical density at 600 nm every 24 hours for 8 days. In addition, growth rates were calculated from the growth curves at the end of the exponential growth phase. Subsequently, I assessed the ability of the non-lethal concentrations to interfere with the adhesion phase, the first step involved in biofilm formation. Shortly, cell suspension supplemented with 0 and non-lethal concentrations of each compound were placed in 96-wells microtiter plates. After incubation of 12 h at 28°C with agitation planktonic cells were removed, adhered cells were washed three

times with distilled water and were stained using 0.1 % of crystal violet. After 20 min the microtiter plates were washed three times with distilled water and after the addition of 200 µL of ethanol the optical density at 600 nm was measured. Lastly, the capability of the selected compounds to interfere with the other steps of biofilm formation was evaluated. Briefly, 7 days old colonies from PW agar plates were collected, resuspended in PD3 medium and 1 mL (OD= 1) was placed in 50 ml of PD3. After 72h, planktonic fraction was discarded and biofilm was collected and resuspended in 5 ml of EDTA 2%.
In planta assays were performed under greenhouse conditions. Briefly, the top of the stem of two months old tobacco plants (*Nicotiana tabacum* 'Petite Havana SR1') was cut keeping only three healthy adult leaves in the lower portion of the plant and 20 µL of cell suspension was injected in each tobacco petiole. The injection of some plants with only buffer (without *X. fastidiosa*) represent the control of the experiment. Different concentrations of zinc oxide nanoparticles (500, 1000, 2000 and 3000 ppm) will be applied to the soil once a week and at specific days post inoculation random petioles will be collected and bacterial population will be quantified via quantitative polymerase chain reaction (qPCR). In addition, to evaluate the effective amount of compounds absorbed by tobacco plants, a ionome quantification will be performed. Lastly, according to their appearance leaves were classified from buffer-inoculated control plants and *X. fastidiosa*-inoculated plants.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The responses of the planktonic growth of the selected *X. fastidiosa* strains in the presence of the different concentrations of compounds showed maximum growth inhibition at the highest concentrations tested (12, 6 and 3 mM) for both salicylic acid and n-acetylcisteine. The concentration of 1 mM of n-acetylcisteine inhibited only the growth of WM 1-1 strain, with a reduction of 33% compared with the control. For both strains, 1 mM of salicylic acid had a low inhibition effects, reducing the growth of 8% and 29% for WM 1-1 and BB08-1 strain respectively, compared with the control. Concentrations below 1 mM did not affect bacterial growth and were selected for the future assays. For zinc oxide nanoparticles, every concentration tested affected the growth of WM 1-1. The same response occurred even for BB08-1 strain, except for the concentration of 10 ppm that highlighted no inhibition effect compared with the control. For this reason, 10 ppm of zinc oxide nanoparticles has been choosen for the subsequent assays. The most promising results of microplate-based adhesion assay revealed that every non-lethal concentration of salicylic acid was able to affect the first step of biofilm formation, with a reduction of cell adhesion up to 60% compared with the control.
Preliminary results obtained from the treatment of 10 ppm of zinc nanoparticles on BB08-1 showed that this concentration affect biofilm formation, reducing protein concentration up to 50% compared with the control.
Greenhouse experiments are taking place and in the following months will be higlighted any differences in terms of bacterial population, leaves appearance and ionome quantification in treated and untreated plants. Preliminary results showed some phytotoxic effects at the highest concentrations tested of zinc oxide nanoparticles (2000 and 3000 ppm).

FUTURE COLLABORATIONS (if applicable)

My collaborative research in the group of Prof. De La Fuente will continue until the end of June 2018. During the following months, I will study in deep the effect of the most promising non-lethal concentrations of the selected compounds on maturation and dispersion phases involved in *Xylella fastidiosa* biofilm. Lastly, the effect of zinc oxide nanoparticles under greenhouse conditions will be evaluated.