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Monitoring of biofilm production in *Xylella fastidiosa* strain De Donno via biochemical signalling modulation

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Abstract: Diffusible lipid species are exploited by bacteria for regulating cell motility, cell-to-cell communication, activation of metabolism and proliferation. The most widely investigated lipid family is represented by diffusible signal factors (DSFs) responsible for quorum sensing. *Xylella fastidiosa* (Xf) uses the DSFs to coordinate genes involved in the expression of virulence and biofilm formation. These moieties are mainly cis-2-unsaturated fatty acids which directly enhance xylem infection and biofilm production, which are the two processes involved in the genesis of severe plant diseases such as the noticeable Olive Quick Decline Syndrome, associated with Xf subsp. *pauca* strain De Donno and affecting olives in the Apulia Region. Here we report the results of studies aiming to identify DSFs molecules of Xf De Donno and exploit strategies for modulating its biofilm formation.

We started inducing DSF expression in *Escherichia coli* using a plasmid vector recombinant for the *rpfF* gene of Xf De Donno and verifying the production of exogenous proteins and fatty acids. We set up extraction, mechanical treatments, and methyl ester derivatisation of the extracted crude oils from Xf and *E. coli* cultures. We compared the GC-MS profiles of fatty acids belonging to the metabolic activity of bacteria harbouring the bare and *rpfF*-recombinant plasmids. Completing previous studies, we speculated on the production of unsaturated fatty acids with a chain length of 12-18 carbon atoms, with α-unsaturated functions. Isolated and treated crude extracted oils obtained from the same bacterial sources, were tested *in vitro* to investigate their phenotypic effect on biofilm growth and the expression of key genes related to surface adhesion, biofilm formation and cell movement.

Furthermore, we set the synthesis of new, no commercially available, cis-2-unsaturated fatty acids with a chemical structure related to the DSFs family, in order to test the *in vitro* alteration of biofilm production in Xf De Donno. The exploited reaction was the stereoselective Still-Gennari olefination which leads to the synthesis of unsaturated fatty acids in cis (Z) conformation starting from commercial aldehydes.

Results of these activities will be presented.

Experimental confirmation that *Xylella fastidiosa* subsp. *pauca*, ST53, does not colonise grapes

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Abstract: *Xylella fastidiosa* is able to colonise a very large number of plant species, but considering each subspecies/phylogenetic clade the number of associated susceptible hosts is significantly reduced. Although strains genetically related most likely share similar host range, using phylogenetic relationships to infer information regarding the potential host

range of new strains is still problematic, and pathogenicity tests remain the only means to assess the capability of a given strain to infect or not a specific plant species. Based on the current European legislative provisions on *X. fastidiosa*, information on the host range of the strain(s) causing an outbreak have regulatory consequences. In this context, we have made efforts to prove experimentally the capability of *X. fastidiosa* subsp. *pauca*, ST53, one of the most virulent European genotypes, to infect grapes, using 23 grape varieties (*Vitis vinifera*) and four rootstocks. Upon needle inoculation, plants were monitored for 18 months, using standand diagnostic methods (qPCR and isolation), supported by vector-transmission tests and observation of thin sections of the inoculated stems, stained using the LIVE/DEAD BacLight kit. qPCR assays on samples collected at the inoculation points (i.p.) and from the distal portions, 6 and 12 months post-inoculation, yielded positive reactions in more than 90% of the i.p., whereas in half of the cultivars scattered amplifications (the majority yielding Cq values > 30) occurred in some replicates at 15–20 cm from the i.p., but none of the apical portions tested positive. Isolations made 18 months after the inoculation, either from mature leaf petioles and stems portions harbouring the inoculation points, failed to recover actively growing colonies. At the same time, microscope observation of the thin sections showed only the presence of aggregates of dead *Xylella*-cells at the i.p. Transmission tests performed using specimens of *Philaenus spumarius* caged on the inoculated grapes produced negative results for both insects and recipient plants. The overall results showed that the bacterium was successfully delivered into the stem of the grapes and bacterial residual could be qPCR-detected even one year after the inoculation, but none of the inoculated cultivars sustained active bacterial multiplication and colonisation.

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Transformation of *Xylella fastidiosa* subspecies *pauca* strain De Donno

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Abstract: *Xylella fastidiosa* subsp. *pauca* strain De Donno has been recently identified as the causal agent of a severe disease affecting olive trees in a wide area of the Apulia Region (Italy). While insights on the genetics and epidemiology of this virulent strain have been gained, the complex network of interactions with the main susceptible host remains to be explored. A fundamental tool for understanding such interactions is the development of bacterial mutants for functional analysis of genes involved in the host recognition, pathogenicity and insect transmission. Experimental studies have demonstrated the natural competence of *X. fastidiosa* in the uptake of exogenous genetic material; a feature exploited for site-specific introduction or deletion of genes through homologous recombination. Nevertheless, numerous studies have shown that several factors may affect *X. fastidiosa* transformation efficiency, including growth rate, twitching motility, sequence similarity, and the presence of Restriction–Modification systems that cleave incoming DNA. On this basis, two different plasmids containing the chromosomal replication origin (oriC) of *X. fastidiosa* and *E. coli* were used to transform *X. fastidiosa* De Donno in order to produce a GFP-expressing and a knockout strain for the *rpfF* gene, a crotonase producing a diffusible signal factor (DSF), involved in the quorum-sensing system. Repeated attempts to exploit natural competence, introducing the donor plasmids into *X. fastidiosa* De Donno failed, highlighting the critical role of genetic diversity in recombination performances of this pathogen. Conversely, GFP and RpfF mutants were successfully obtained by co-electroporation in the presence of an inhibitor of the Type I R-M system, that had been proved to impact the stable acquisition of foreign DNA by *X. fastidiosa* subsp. *fastidiosa*. Availability of mutants for one of the most virulent strains of *X. fastidiosa* opens for new explorations of host–microbe interactions, important to elucidate mechanisms