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BOOK OF ABSTRACTS

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S4-P7

Methylases and methylation in gene regulation of *Pseudomonas syringae.*

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DNA methylation carried out by methyltransferases not associated with restriction enzymes, known as orphan methyltransferases, is an epigenetic mechanism widely spread in bacteria and archea. These orphan methylases show motif specificities and methylation patterns that support their function in gene regulation and DNA replication[1]. In *E. coli* and *Salmonella*, this mechanism has been related to the regulation of the initiation of replication, DNA mismatch repair and transcription. DNA methylation is also involved in generating phenotypic heterogeneity within clonal populations in these species, with strong implications for virulence[2]. However, little is known about the role of methylation in *Pseudomonas syringae*. *P. syringae* is a relevant phytopathogenic bacteria, responsible for a great variety of plant diseases and with a huge impact in crop production worlwide. This bacteria is also used as a model for the study of plant-pathogen interactions[3]. In order to unveil the importance of methylation in *P. syringae,* we decided to address the characterization of this epigenetic process in this pathogenic bacteria. For that, we have studied the methylases identified in *P. syringae,* and established its methylome. Regarding the methylome, we have identified several methylation motifs.

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[1]Blow M. *et al.*, 2016. PLoS Genet, 12(2), 1-28.
[2]Adhikari S. and Curtis P.D., 2016. FEMS Microbiol. Rev., 40(5), 575-591.
[3]Xin X. *et al.*, 2018. Nat. Rev. Microbiol., 16(5), 316-328.

S4-P8

Immune Recognition of the Secreted Serine Protease ChpG Restricts the Host Range of *Clavibacter michiganensis* from eggplant varieties

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Bacterial wilt and canker caused by *Clavibacter michiganensis* (*Cm*) inflicts considerable damage in tomato-growing regions around the world. *Cm* harbors a narrow host range and can cause disease in tomato but not in most eggplant varieties. The pathogenicity of *Cm* is dependent on secreted serine proteases of the Pat-1 family, that are encoded on the *chp/tomA* pathogenicity island (PI), and the pCM2 plasmid. Screening combinations of PI deletion mutants and plasmid-cured strains found that *Cm*-mediated hypersensitive response (HR) in eggplant is dependent on the *chp/tomA* PI. Singular reintroduction of the four PI encoded Pat-1 family proteases into *Cm*ΔPI identified that the HR is elicited by the Pat-1 family protease ChpG. Eggplant leaves infiltrated with *chpG* marker exchange mutant (*CmΩchpG*) did not display HR, and infiltration of purified ChpG protein elicited immune responses in eggplant but not in *Cm*-susceptible tomato. Virulence assays found that while wild-type *Cm* and *CmΩchpG* complemented strains were nonpathogenic on eggplant, *CmΩchpG* caused wilt and canker symptoms. Additionally, bacterial populations in *CmΩchpG*-inoculated eggplant stem tissues was ~1000 folds higher than wild-type and *CmΩchpG* complemented *Cm* strains. Alanine substitution of Serin231 of the putative serine protease catalytic triad eliminated the ability of purified ChpG protein to elicit HR on eggplant and introduction of $ChpG_{S231A}$ into *CmΩchpG* did not affect its ability to cause disease, indicating that protease activity is required for immune recognition of ChpG. Our study identified ChpG as a novel avirulence protein that is recognized in eggplant and restricts the host range of *Cm*.