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## **IMPLICATION OF C-DI-GMP IN PHYTOPATHOGENIC BACTERIA *Xanthomonas vesicatoria***

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*Xanthomonas vesicatoria* (Xv) is a member of a complex of species that causes bacterial spot on tomato (*Solanum lycopersicum*), a disease distributed worldwide. At the stage of infection several virulence factors are involved in causing the disease. Flagella and pili are important for attachment to surfaces leading to biofilm formation. Also, the exopolysaccharide xanthan contributes to bacterial stress tolerance and enhances biofilm formation by enabling bacteria to adhere to the plant surfaces. Finally, when bacteria come into contact with the plant, extracellular enzymes help degrade host plant cell walls, contributing to virulence. Bis-(3'5')-cyclic dimeric guanosine monophosphate (c-di-GMP) is a second messenger involved in bacterial virulence and plays a role in regulating a number of processes as the ones mentioned before. Two types of enzymes are responsible for regulating c-di-GMP levels: diguanylate cyclases (DGC), which synthesize c-di-GMP, and phosphodiesterases (PDE), which degrade c-di-GMP. To test the implication of different levels of c-di-GMP in Xv virulence, the Xv strain BNM 208 was transformed with plasmids coding for a DGC and PDE proteins, in order to obtain Xv strains with high (208-DGC) and low (208-PDE) c-di-GMP levels, respectively. Assays for characterization of the phenotypic behavior in response to c-di-GMP levels were carried out as follows: we analyzed motility (swimming and swarming) in soft agar plates by measuring bacteria diffusion; the production of extracellular enzymes were determined by measuring the degrading halo; survival to stress agents were assessed by a quantitative method (UV radiation and heat-shock) counting CFU/ml, and by a qualitative method in agar plates (detergent and hydrogen peroxide). For biofilm formation violet crystal method was used and the biofilm architecture was observed at a confocal microscope. To assess xanthan weight, precipitation with ethanol was carried out. In planta assays were carried out by plant immersion in

bacterial culture. The 208-DGC strain presented reduced motility (swimming and swarming), less cellulase activity, higher adhesion, less xanthan weight and less survival to stress agents, compared to 208-PDE strain. Also, when infected tomato plants, 208-DGC strain presented less infection percentage compared to 208-PDE strain. Our analysis showed that c-di-GMP is involved in the regulation of important virulence traits in *Xv*. However, the c-di-GMP mediate-responses in *Xv* is still poorly understood, and even within the same species it might act differently. This study contributes in unveiling the network of responses mediated by c-di-GMP for a better understanding of cellular and molecular mechanisms involving this metabolite.

Palabras clave: *Xanthomonas* - c-di-GMP - tomato