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Non-Polar Knockouts Of Genes Involved To Motility And Virulence In The Phytophatogen *Xanthomonas axonopodis* pv *citri*

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The survival of bacteria in different environment depends on its capacity to perceive and adapt to its environment. For this the bacterial cell regulates gene expression and metabolism in order to modify its physiological traits and structural apparatus. In this work we have produced a number of non-polar mutants in *Xanthomonas axonopodis* pv *citri* (Xac) and studied their phenotypes. The non-polar mutants were constructed using the pNPTS vector, which has the kanamycin resistance and *sac*B gene (levansucrase) as positive and negative selection markers for the first and second recombination events. The mutants for each gene were confirmed by PCR. The *rpf* (Regulator of Pathogenicity Factors) mutants presented decreased motility and reduced production of virulence factors (proteases and endoglucanases). These mutants form aggregates when grown in L medium. The phenotype of the *rpf*F mutant can be reverted to the wild type phenotype (reduce the aggregation) by the addition of exogenous DSF. Furthermore, the aggregative phenotype as the *rpf* mutant was similar to that observed when the gene XAC0424 that codes for a diguanilate cyclase was overexpressed in the *Xac* cells. These results suggest the aggregative behavior in *Xanthomonas* can be controlled by cyclic diGMP (c-diGMP) concentration in the cell. Nevertheless the regulation of this process by c-diGMP and the precise molecular targets of c-diGMP have not yet been identified. We identified others genes related to RNA binding proteins, c-diGMP signaling, ppGpp production and degradation and tow-component signalling that may be important points for regulation of virulence determinants and motility of *Xanthomonas axonopodis* pv citri.

Key words: *Xanthomonas*, *rpf*, cyclic diGMP, virulence factors and motility.
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