Genes and phenotypes controlled by the Gac-rsm pathway in *Pseudomonas syringae* pv. tomato DC3000

Pseudomonas syringae pv. tomato (Pto) DC3000 is a model bacteria used to study plant-phytopathogen interactions (Preston, 2000). It causes bacterial speck on tomato thanks to its large repertoire of effectors that are secreted through the type III secretion system and the phytotoxin coronatine, which disrupts signalling mediated by jasmonic acid and stimulates stomatal opening, allowing the entry of bacteria to the apoplast. In addition, Pto possesses other tools that contribute to pathogenicity, as flagella and biosurfactants, which facilitate its movement, or exopolysaccharides, that prevent desiccation.

It was previously established that the GacS/GacA two component system functions as a global regulator in Pto DC3000 controlling a large variety of factors (Chatterjee, *et al*., 2003, Ge, *et al*., 2019, Nakatsu, *et al*., 2019, O'Malley, *et al*., 2019a; 2019b) that cause drastic changes in bacterial behavior affecting virulence, multiplication in plant, hypersensitive response (HR) induction efficiency, pigment and N-acyl-homoserine lactone production, and swarming. Pto DC3000 possesses seven small RNAs controlled by GacA and five CsrA/RsmA-like RNA binding proteins (Heeb *et al*., 2006; Kulkarni *et al*., 2006; Moll *et al*., 2010; Ferreiro *et al*., 2018; 2021; Ferreriro & Gallegos, 2021; Ge, *et al*., 2019).

The overall objective of the present work is the characterization of the Pto DC3000 Gac-Rsm pathway and the elucidation of its physiological role. The specific objectives are the study of the expression and regulation of:

1. The Rsm regulatory proteins.
2. The Rsm regulatory RNAs.
3. The target mRNAs of the Gac-Rsm pathway.

This study will be tackled from a multidisciplinary point of view, using new methodologies like bioinformatics, genomic, proteomic, combined with cellular and molecular approaches. Mutant strains in the component of the Gac-Rsm pathway and target genes will be constructed and phenotypically characterised. We will focus on phenotypes important for plant colonization and symptom development, like swimming, swarming, virulence factor production, biosurfactant, EPS or siderophore synthesis. Also, gene expression will be analysed under diverse conditions (laboratory media, *in planta*, etc.) by different *in vivo* and *in vitro* methods.

Chatterjee, A., Cui, Y., Yang, H., Collmer, A., Alfano, J.R., Chatterjee, A.K. 2003. GacA, the response regulator of a two-component system, acts as a master regulator in *Pseudomonas syringae* pv. tomato DC3000 by controlling regulatory RNA, transcriptional activators, and alternate sigma factors. Mol Plant Microbe Interact 16:1106-1117.

Ferreiro, M.D., Nogales, J., Farias, G.A., Olmedilla, A., Sanjuán, J., Gallegos, M.T. 2017. Multiple CsrA proteins control key virulence traits in *Pseudomonas syringae* pv. tomato DC3000. Mol Plant Microbe Interact 31: 525-536.

Ferreiro, M.D., Behrmann, L.V., Corral, A., Nogales, J., and Gallegos, M.T. (2021) Exploring the expression and functionality of the rsm sRNAs in *Pseudomonas syringae* pv. tomato DC3000, RNA Biol. 10.1080/15476286.2020.1871217.

Ferreiro, M.D., Gallegos, M.T. 2021. Distinctive features of the Gac-rsm pathway in plant-associated *Pseudomonas*. Environ. Microbiol. 23: 5670-5689.

Ge, Y., Lee, J.H., Liu, J., Yang, H.W., Tian, Y., Hu, B., and Zhao, Y. (2019) Homologues of the RNA binding protein RsmA in *Pseudomonas syringae* pv. tomato DC3000 exhibit distinct binding affinities with non-coding small RNAs and have distinct roles in virulence, Mol. Plant Pathol. 20: 1217-1236.

Heeb, S., Kuehne, S.A., Bycroft, M., Crivii, S., Allen, M.D., Haas, D., Camara, M., Williams, P. 2006. Functional analysis of the post-transcriptional regulator RsmA reveals a novel RNA-binding site. J Mol Biol 355:1026-1036.

Kulkarni, P.R., Cui, X., Williams, J.W., Stevens, A.M., Kulkarni, R.V. 2006. Prediction of CsrA-regulating small RNAs in bacteria and their experimental verification in *Vibrio fischeri*. Nucleic Acids Res 34:3361-3369.

Moll, S., Schneider, D.J., Stodghill, P., Myers, C.R., Cartinhour, S.W., Filiatrault, M.J. 2010. Construction of an *rsmX* co-variance model and identification of five *rsmX* non-coding RNAs in *Pseudomonas syringae* pv. tomato DC3000. RNA Biol 7:508-516.

Nakatsu, Y., Matsui, H., Yamamoto, M., Noutoshi, Y., Toyoda, K., and Ichinose, Y. (2019) Quorum-dependent expression of rsmX and rsmY, small non-coding RNAs, in Pseudomonas syringae, Microbiol. Res. 223-225: 72-78.

O'Malley, M.R., Chien, C.F., Peck, S.C., Lin, N.C., and Anderson, J.C. (2019a) A revised model for the role of GacS/GacA in regulating type III secretion by *Pseudomonas syringae* pv. tomato DC3000, Mol. Plant Pathol. 21: 139-144.

O'Malley, M.R., Weisberg, A.J., Chang, J.H., and Anderson, J.C. (2019b) Re-evaluation of a Tn5::gacA mutant of *Pseudomonas syringae* pv. tomato DC3000 uncovers roles for uvrC and anmK in promoting virulence, PLoS One 14: e0223637.

Preston, G.M. 2000. *Pseudomonas syringae* pv. tomato: the right pathogen, of the right plant, at the right time. Mol Plant Pathol 1:263-275.

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