

**THE HD-GYP DOMAIN OF RPF G MEDIATES A DIRECT LINKAGE BETWEEN THE RPF QUORUM-SENSING PATHWAY AND A SUBSET OF DIGUANYLATE CYCLASE PROTEINS IN THE PHYTOPATHOGEN *XANTHOMONAS AXONOPODIS PV CITRI*.**

Maxuel O. Andrade<sup>1</sup>, Marcos C. Alegria<sup>1</sup>, Cassia Docena<sup>1</sup>, Cristiane R. Guzzo<sup>1</sup>, Maria C.P. Rosa<sup>1</sup>, Carlos H.I. Ramos<sup>2,3</sup>, and Chuck S. Farah<sup>1</sup>

<sup>1</sup>IQ-USP; <sup>2</sup>Centro de Biologia Molecular Estrutural, LNLS; <sup>3</sup>IB-UNICAMP.

We have used the yeast two-hybrid system and direct *in vitro* assays to identify interactions involving the Rpf group of proteins that constitute a quorum-sensing signal transduction pathway *Xanthomonas spp.* This pathway consists of RpfF, necessary for the production of the autoinducer “diffusible signaling factor” (DSF), and the two component system made up of RpfC and RpfG which are necessary for the DSF-dependent expression of genes responsible for the production of extracellular pathogenicity factors as a function of bacterial cell density. We show that RpfC, a modular protein consisting of an N-terminal transmembrane domain, a histidine kinase domain, a response-regulator domain and a C-terminal histidine phosphotransfer domain interacts both with RpfG, a protein consisting of an N-terminal response regulator domain and a C-terminal HD-GYP domain and with RpfF. We also show that RpfC interacts with the only known homolog of “conditioned medium factor” (CMF), which is involved in quorum-sensing in the slime mold *Dictyostelium discoideum* under conditions of nutritional stress. Finally we show that the recently characterized phosphodiesterase HD-GYP domain of RpfG interacts directly with GGDEF domain-containing proteins coded by the *Xanthomonas axonopodis pv. citri* genome. In other bacteria, GGDEF domains have been shown to possess diguanylate cyclase activity necessary for the production of cyclic diGMP, an important second messenger involved in the regulation of complex bacterial processes including biofilm production, virulence and motility. These results demonstrate a direct physical linkage between quorum-sensing and cyclic di-GMP signaling pathways in bacteria.

Keywords: quorum sensing, virulence, c-diGMP, HD-GYP, GGDEF