Mapping and validation of *Xanthomonas citri* subsp *citri* genes regulated by putative plant-inducible promoter box (PIP-box)

F.M.S. Carvalho[1,2], J.C.F. Oliveira[1,3], M.L. Laia[1,4], T.R. Jacob[1,2], R.M. Ferreira[1], M.I.T. Ferro[1], R.I.D. Tezza[1,6], S.M. Zingaretti[1,5], C.F. Silva[1] and J.A. Ferro[1,6]

[1]Laboratório de Bioquímica e Biologia Molecular, Departamento de Tecnologia, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brasil
[2]Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil
[3]Departamento de Ciências Biológicas, Universidade Federal de São Paulo, Diadema, SP, Brasil
[4]Departamento de Engenharia Florestal, Faculdade de Ciências Agrárias, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG, Brasil
[5]Unidade de Biotecnologia, Universidade de Ribeirão Preto, Ribeirão Preto, SP, Brasil
[6]Centro de Recursos Biológicos e Biologia Genômica, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brasil

Corresponding author: F.M.S. Carvalho
E-mail: flaviasc2@hotmail.com

Genet. Mol. Res. 15 (2): gmr.15028428
Received: January 12, 2016
Accepted: March 11, 2016
Published: May 6, 2016
DOI http://dx.doi.org/10.4238/gmr.15028428

ABSTRACT. Citrus canker, caused by the Gram-negative bacterium *Xanthomonas citri* subsp *citri* (Xac), is a major disease affecting citriculture worldwide, because of the susceptibility of the host and the

lack of efficient control methods. Previous studies have reported that some genes of phytopathogenic bacteria possess a consensus nucleotide sequence (TTCGC...N15...TTCGC) designated the "plant-inducible-promoter box" (PIP box) located in the promoter region, which is responsible for activating the expression of pathogenicity and virulence factors when the pathogen is in contact with the host plant. In this study, we mapped and investigated the expression of 104 Xac genes associated with the PIP box sequences using a macroarray analysis. Xac gene expression was observed during *in vitro* (Xac grown for 12 or 20 h in XAM1 induction medium) or *in vivo* (bacteria grown in orange leaves for 3 to 5 days) infection conditions. Xac grown in non-induction NB liquid medium was used as the control. cDNA was isolated from bacteria grown under the different conditions and hybridized to the macroarray, and 32 genes differentially expressed during the infection period (*in vitro* or *in vivo* induction) were identified. The macroarray results were validated for some of the genes through semi-quantitative RT-PCR, and the functionality of the PIP box-containing promoter was demonstrated by activating β-glucuronidase reporter gene activity by the PIP box-containing promoter region during Xac-citrus host interaction.

Key words: PIP-Box promoter; Gene reporter β-glucuronidase; Citrus canker; *Xanthomonas citri*; XAMI induction medium

