

Describing the roles of the Type 6 Secretion System in *Xanthomonas citri* subsp *citri* physiology: a gene expression analysis approach

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Abstract

The phytopathogen *Xanthomonas citri* subsp *citri* (Xac) is responsible for big economic losses in agriculture. Several important processes to bacteria's survival are mediated by the translocation of macromolecules to the extracellular milieu or to the target cells. The type 6 Secretion System (T6SS) is a recently described mechanism to secrete proteins and is usually associated to interbacterial competition, but in Xac this function is known to be dependent of another type of secretion system, the type 4 (T4SS). In this work, we have analyzed the gene expression patterns of some T6SS core protein genes during epiphytic growth and infection process in Xac in order to gain further insights in T6SS function in this bacterium. Our results show that these genes are induced during epiphytic growth but tightly repressed when Xac is growing inside plant leaf tissue and promoting the disease. These results demonstrate that the T6SS is not involved in pathogenesis, but may be required in some important aspects of Xac lifestyle, such as biofilm formation, adhesion to biotic surfaces/or interbacterial competition.

Key words: *Xanthomonas citri*, Secretion Systems, Type 6 Secretion System.

Introduction

A range of macromolecules is actively exported to the extracellular milieu and target cells during bacterial cell cycle. Gram-negative bacteria translocate proteins by distinct Secretion Systems machineries (named type 1 to 6) that differs in protein components, structure, secreted substrates and function.¹ Xac causes citrus canker and is responsible for big economic losses in agriculture. The most aggressive strain, Xac306, possesses at least one secretion system of each type.² T6SS was recently described³ and is usually involved in interbacterial competition as well as in pathogenesis and biofilm formation in other bacteria^{4,5,6,7}. In Xac306, a T4SS was described as required for interbacterial competition⁸, a role played by the T6SS in other bacterial species. The focus of our studies is the identification of the function, secreted substrates and regulation of the Xac T6SS. At the present work, we describe the conditions that induce or repress the expression of genes encoding essential components of the T6SS structure. The characterization of the conditions in which this secretion system is expressed will give new insights into its role for Xac physiology.

Results and Discussion

The expression of core protein genes of the Xac T6SS (*xac4147*, *xac4140* and *xac4124*) was analyzed by qRT-PCR in two different conditions: during epiphytic growth and during the infection process. Bacteria were inoculated in leaf surfaces of sweet orange plants (*Citrus sinensis* Osbeck) by spraying of cultures grown to an OD_{600nm}=1.0 (epiphytic growth) or by injection inside plant tissue (infectious process). RNA was extracted in distinct times after inoculation and reverse

transcribed into cDNA for gene expression analysis by qRT-PCR using the housekeeping genes *rpoB* and *rpoC* as normalizers⁹. All three genes analyzed showed a significant induction in expression during epiphytic growth of up to 10 times. However, T6SS gene expression was highly repressed in bacteria grown inside host leaves, showing about a 40x average decrease after inoculation. A similar expression pattern was also observed in T4SS genes, except by a less pronounced repression during infection (about 3x), which suggests that these two secretion systems might have complementary functions in interbacterial competition and/or biofilm formation in Xac.

Conclusions

This work shows that Xac T6SS may not be required for the infectious process of Xac. In addition, our results suggest that this secretion system is implicated in other conditions required for Xac growth in the environment, which can be related to biofilm formation as well as interbacterial competition.

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¹Gerlach, R.; Hensel, M. *International Journal of Medical Microbiology*. **2007**, 297, 401-415.

²da Silva, A. C. R., et al. *Nature*. **2002**, 407, 459-463.

³Pukatzki, S. et al. *PNAS* **2006**, 103, 1528-1533.

⁴Hood, R. et al. *Cell*, **2011**, 7, 25-37

⁵Jani, A.; Cotter, P. *Cell Host and Microbe* **2010**, 8, 2-6.

⁶Schell, M. et al. *Molecular Microbiology* **2007**, 64, 1466-1485

⁷Weber, B. et al. *Environmental Microbiology* **2009**, 11, 3018-3028

⁸Souza, D. et al. *Nature Communications* **2015**, 6, 6453

⁹Jacob, T. R. et al. *Biotechnology Letters*. **2011**, 33, 1177-1184