REGULATION OF FIMBRIAL GENES BY SIGMA54 FACTOR IN *Xylella fastidiosa*

José F. da Silva Neto[1], Tie Koide[2], Suely L. Gomes[2], Marilis V. Marques[1]
[1]Departamento de Microbiologia, ICB, USP, SP, Brazil; [2]Departamento de Bioquímica, IQ, USP, SP, Brazil

Xylella fastidiosa is a Gram-negative phytopathogenic bacterium that is the causative agent of important diseases, including citrus variegated chlorosis (CVC). In this work, we have investigated the role of sigma54 and its involvement in the regulation of fimbrial biogenesis in *X. fastidiosa*. The gene encoding the sigma54 factor was cloned from strain J1a12 and an *rpoN* null mutant was constructed. Analysis of global gene expression profile by microarray comparing the wild type and *rpoN* mutant strains showed that few genes exhibited differential expression. At least one gene, *pilA*, which encodes the structural pilin protein of type IV fimbriae, showed decreased expression in the *rpoN* mutant whereas an operon encoding proteins of type I fimbriae were only twofold more expressed in the mutant. Quantitative real time RT-PCR (qRT-PCR) analysis confirmed that *pilA* transcript was significantly reduced in the *rpoN* mutant. Genome sequence analysis of *X. fastidiosa* revealed five paralogues of *pilA*. Microarray and qRT-PCR data demonstrated that only one transcript was significantly affected in the *rpoN* mutant. The transcriptional start site of *pilA* was determined by primer extension. Upstream of the start site, we identified a canonical sigma54-dependent promoter. Using a quantitative assay, we demonstrated that the *rpoN* mutant made more biofilm than the wild type strain. These results to indicate that sigma54 regulates negatively biofilm formation probably due differential regulation of genes involved in type IV and type I fimbrial biogenesis.

Keywords: sigma54 factor; Type IV pili; biofilm ; *Xylella fastidiosa*
Supported by FAPESP and CNPq