SURROGATE GENETICS FOR *XYLELLA FASTIDIOSA*

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INTRODUCTION

Xylella fastidiosa presents a formidable challenge to the molecular geneticist. Methods for the basic operations of genetic exchange, mutant isolation, and complementation are in early stages of development. The slow generation time, poor plating efficiency and requirement for complex culture media are further complications. Surrogate genetics (Maloy and Zahrt 2000) provides a means to at least partially bypass these challenges. Here, one creates a hybrid organism, transplanting genes of interest from the poorly-studied species (e.g., *Xylella fastidiosa*) into a well-studied surrogate host (e.g., *Escherichia coli*). Given sufficiently related hosts, one expects the transplanted genes to function in the surrogate essentially as they do in the original. One may then exploit the advantageous properties of the surrogate to perform a large number of experiments, making and discarding hypotheses to define various aspects of gene function. Once gene function in the surrogate has been thoroughly explored, one can perform a focused set of experiments, informed by the results from the surrogate, to examine function in the native host. The use of *E. coli* as a surrogate host for studying gene regulation would open a range of experimental approaches that are currently unavailable in *X. fastidiosa*, and lead to more rapid advances in understanding the control of key pathogenicity determinants. We are analyzing the transcriptional regulation determinants for genes whose products may be involved in pathogenesis (e.g., *pil* genes, encoding type IV pili) as well as "housekeeping" genes involved in central metabolism (e.g., amino acid biosynthesis).

OBJECTIVES

1. Apply bioinformatics to evaluate transcription control signals *in silico* for *X. fastidiosa* 9a5c
2. Construct and characterize a Φ(*pilA-lacZ*) operon fusion in *E. coli*
3. Construct and characterize a Φ(*glnA-lacZ*) operon fusion in *E. coli*

RESULTS AND CONCLUSIONS

A first approach to defining transcriptional regulatory mechanisms in *X. fastidiosa* is to examine visually the upstream nucleotide sequences of genes whose regulation has been well-studied in other organisms. Common regulatory strategies will be revealed by common features in the sequences. Our initial analysis has focused on the *trp* and *his* operon transcription attenuation control regions which in enterobacteria and other species contain easily-recognized sequence features: regulatory leader peptide coding regions that are rich in codons for the regulatory aminoacyl-tRNA; stem-loop structures that serve as factor-independent transcription terminators; and alternative stem-loop antiterminator structures. However, as revealed by the genome sequence of *X. fastidiosa* strain 9a5c (Simpson et al. 2000), the *X. fastidiosa hisGDCBHAFI* biosynthetic operon upstream regulatory sequence exhibits no leader peptide or terminator structures. Therefore, *his* operon expression in *X. fastidiosa* is regulated by a mechanism other than transcription attenuation. The *X. fastidiosa trp* biosynthetic genes are not organized in a single *trpE(G)DC(F)BA* operon as in *E. coli*, but rather in three noncontiguous operons: *trpEGDC*, *trpF*, and *trpBA*, in an arrangement mimicking that of *Pseudomonas aeruginosa*. Again, however, the *X. fastidiosa trp* gene upstream regions do not contain apparent regulatory regions similar to those for controlling *trp* gene expression in either *E. coli* or *P. aeruginosa*. Thus, regulation of these amino acid biosynthetic pathways must occur through other mechanisms in *X. fastidiosa*.

Environmental and genetic controls of exopolysaccharide (EPS) biosynthesis remain largely undefined (Rodrigues da Silva et al. 2001). The laboratory of Michael Daniels (John Innes Centre) has identified a cluster of linked *Xan. campestris* pv. campestris regulatory genes, mutations in which affect production of several extracellular enzymes, including endoglucanases and proteases, along with EPS. The *rpfC* and *rpfG* genes initially were thought most likely to encode direct transcriptional regulators of pathogenicity gene expression. However, more recent analysis indicates that the RpfG protein is probably not a direct (DNA-binding) regulator of *gum* gene expression (Slater et al. 2000). We constructed a Φ(*gumB-lacZ*) operon fusion in *E. coli*, in order to use LacZ expression as a measure of *gumB* promoter activity. However, this construct expressed only low levels of LacZ enzyme. Given the uncertain nature of *gum* operon regulation, we elected to turn our immediate attention to study genes whose expression is more readily predicted from sequence inspection.

We therefore chose to study the regulation of *pil* gene expression. These genes control the formation of type IV pili in a variety of organisms, and are required for gliding motility, adhesion and pathogenesis (Winther-Larsen and Koomey 2002; Shi and Sun, 2002). Expression of *pilA* structural genes requires a specialized RNA polymerase specificity determinant (σ^{54}) which recognizes a strongly-conserved -12/-24 nucleotide sequence. One of two *pilA* homologs (XF2542) in *X. fastidiosa* contains a σ^{54}-dependent promoter. We constructed a Φ(*pilA-lacZ*) operon fusion in *E. coli*, and observed that it expressed detectable levels of LacZ enzyme. We also cloned the regulatory *pilSR* genes (XF2546 and XF2545) from *X. fastidiosa*. However, we have not yet observed a *pilR*-dependent increase in LacZ synthesis, indicating that the PilSR regulators may not function well in *E. coli*.

Unpublished work of others indicates that σ^{54}-dependent activators from other species do not function well with *E. coli* RNA polymerase. To approach this question directly, we are currently studying expression of the *glnA* gene encoding glutamine synthetase (XF1842). This is the best-studied σ^{54}-dependent gene in *E. coli*, and the *X. fastidiosa glnA* upstream regulatory region is similar to that of *E. coli*. Furthermore, *X. fastidiosa* encodes the NtrB-NtrC sensor-regulator system for controlling *glnA* gene expression (XF1849 and XF1848). Because *E. coli* also encodes NtrB-NtrC, we will be able to evaluate *glnA* expression in response to both the *X. fastidiosa* and the *E. coli* regulatory proteins.

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