

## SURROGATE GENETICS FOR *XYLELLA FASTIDIOSA*

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### ABSTRACT

Our long-term goal is to understand the specificity determinants that govern transcription initiation in *Xylella fastidiosa* (*X. fastidiosa*). Our approach is to use *Escherichia coli* as a surrogate host for reconstituting regulated transcriptional control circuits. This allows us to identify and focus on *X. fastidiosa*-specific features. Our initial studies are aimed at understanding the transcriptional regulation of type IV pilus synthesis.

### 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 and poor plating efficiency are further complications. Surrogate genetics (Maloy & 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., *X. fastidiosa*) into a well-studied surrogate host (e.g., *E. 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., type IV pili) as well as "housekeeping" genes involved in central metabolism (e.g., glutamine synthetase, involved in amino acid biosynthesis and nitrogen assimilation).

### OBJECTIVES

1. Reconstitute regulated  $\Phi(glnA-lacZ)$  expression in *E. coli*.
2. Reconstitute regulated  $\Phi(pilA-lacZ)$  expression in *E. coli*.
3. Begin analyzing housekeeping gene promoter structure in *X. fastidiosa*.

### RESULTS

We chose first to study the regulation of *pil* gene expression from *X. fastidiosa* Temecula (Van Sluys et al., 2003). These genes control the formation of type IV pili in a variety of organisms, where they are required for gliding motility, adhesion, transformation and pathogenesis (Winther-Larsen & Koomey, 2002; Shi & Sun, 2002). Expression of *pilA* structural genes requires a specialized RNA polymerase specificity determinant ( $\sigma^{54}$ ), which recognizes a strongly conserved -12/-24 nucleotide sequence. One of two *pilA* homologs (XF2542) in *X. fastidiosa* contains a  $\sigma^{54}$ -dependent promoter. We constructed a  $\Phi(pilA-lacZ)$  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*.

Recent work of others indicates that a  $\sigma^{54}$ -dependent activator from another species does not function well with *E. coli* RNA polymerase (Richard et al., 2003). To approach this question directly, we are currently studying expression of the *glnA* gene encoding glutamine synthetase (XF1842). This is the best-studied  $\sigma^{54}$ -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 are able to evaluate *glnA* expression in response to both the *X. fastidiosa* and the *E. coli* regulatory proteins. We found that the *X. fastidiosa* *glnA* promoter functions well in *E. coli* when activated by the *E. coli* NtrB-NtrC proteins. This confirms the

identity of this promoter, and establishes its mode of regulation. However, the *X. fastidiosa* NtrB-NtrC proteins did not activate this promoter in *E. coli*. We hypothesize that the NtrC protein must make a species-specific contact with RNA polymerase, perhaps via the  $\sigma^{54}$ -subunit. Experiments to evaluate this idea are under way.

## CONCLUSIONS

We know little about the specificity determinants for regulated transcription initiation in *X. fastidiosa*. RNA polymerase interactions with transcriptional activators such as the NtrC and PilR proteins may involve species-specific protein-protein interactions. Furthermore, the structure of general "housekeeping gene" promoters is not known. Our studies to define these specificity determinants will allow more insightful predictions of gene expression and function.

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***Section 3A:***  
***Xylella fastidiosa Epidemiology***  
***and Biological Control***

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