SURROGATE GENETICS FOR *XYLELLA FASTIDIOSA*: REGULATION OF EXOPOLYSACCHARIDE AND TYPE IV PILUS GENE EXPRESSION

Project Leader:
Valley Stewart
Section of Microbiology
Division of Biological Sciences
University of California
Davis, CA 95616

Cooperator:
Bruce Kirkpatrick
Department of Plant Pathology
College of Agricultural & Environmental Sciences
University of California
Davis, CA

INTRODUCTION

Xylella fastidiosa presents a formidable challenge to the molecular geneticist. There are no published methods available for the basic operations of genetic exchange, mutant isolation, and complementation. The slow generation time, poor plating efficiency and requirement for complex culture media 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., *Xyella 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., *gum* genes, encoding exopolysaccharide; and *pil* genes, encoding type IV pili) as well as "housekeeping" genes involved in central metabolism (e.g., amino acid biosynthesis). During infection, *X. fastidiosa* produces an extracellular matrix that is hypothesized to contribute significantly to disease symptoms (see Bevan, 2000). The *X. fastidiosa* 9a5c genome encodes a *gumBCDEFHJK* operon homologous to the corresponding *Xanthomonas campestris* operon (Simpson et al., 2000). Note however that *Xan. campestris* pv. campestris *gum* null mutants exhibit at most a 50% reduction in virulence index when assayed on cabbage (Katzen et al., 1998). Type IV pili are responsible for twitching motility, natural transformation, and adherence in various species. The *X. fastidiosa* 9a5c genome encodes the various *pil* genes necessary for synthesis, secretion and assembly of type IV pili (Simpson et al., 2000).

OBJECTIVES

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

RESULTS AND CONCLUSIONS

A first approach to defining transcriptional regulatory mechanisms in *X. fastidiosa* is to visually examine the upstream nucleotide sequences of genes whose regulation has been well studied in other organisms. Common features in the sequences will reveal common regulatory strategies. 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, 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 (reviewed by Becker et al., 1998). 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 (Slater et al., 1999) were thought to likely 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 chose therefore to study the regulation of *pil* gene expression. These genes control the formation of type IV pili in a variety of organisms, and the regulatory mechanisms have been studied in *P. aeruginosa* (Mattick et al., 1996) among others. It is hypothesized that these pili (fimbriae) are involved in adhesion to the gut and mouthparts of the insect vector (Bevan, 2000). 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 (XF 2546-2545) from *X. fastidiosa*. The presence of these genes resulted in an approximately twofold increase in LacZ expression, suggesting that they may have activated the *pilA* promoter. Our current experiments are designed (1) to demonstrate that Φ (*pilA-lacZ*) expression requires σ^{54}; and (2) to optimize the expression of the *pilSR* genes in *E. coli*. Our goal is to observe a significant PilR-dependent stimulation of Φ (*pilA-lacZ*) expression. If this is successful, we can then employ surrogate genetics to better understand the control of type IV pilin synthesis.

REFERENCES

Becker, A., F. Katzen, A. Pühler, and L. Ielpi. 1998. Xanthan gum biosynthesis and application: a biochemical/genetic perspective. Appl. Microbiol. Biotechnol. 50:145-152.

Bevan, M. 2000. The bugs from Brazil. Nature 406:140-141.

Katzen, F., D. U. Ferreiro, C. G. Oddo, M. V. Ielmini, A. Becker, A. Pühler, and L. Ielpi. 1998. *Xanthomonas campestris* pv. campestris *gum* mutants: effects on xanthan biosynthesis and plant virulence. J. Bacteriol. 180:1607-1617.

Maloy, S., and T. Zahrt. 2000. Surrogate genetics: the use of bacterial hybrids as a genetic tool. Methods 20:73-79.

Mattick, J. S., C. B. Whitechurch, and R. A. Alm. 1996. The molecular genetics of type-4 fimbriae in Pseudomonas aeruginosa – a review. Gene 179:147-155.

Simpson, A. J. G., et al. 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. Nature 406:151-158.

Slater, H., A. Alvarez-Morales, C. E.Barber, M. J. Daniels, and M. Dow. 2000. A two-component system involving an HD-GYP domain protein links cell-cell signalling to pathogenicity gene expression in *Xanthomonas campestris*. Mol. Microbiol. 38:986-1003.

Slater, H., C. E.Barber, A. Alvarez-Morales, M. J. Daniels, and M. Dow. 1999. A specialised two-component system links cell-cell signalling to pathogenicity gene expression in the phytopathogen *Xanthomonas campestris*. Unpublished DNA sequence, accession AJ251547.