THE ROLES THAT DIFFERENT PILI CLASSES IN *XYLELLA FASTIDIOSA* PLAY IN COLONIZATION OF GRAPEVINES AND PIERCE'S DISEASE PATHOGENESIS.

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Reporting Period: The results reported here are from work conducted September 2007 to February 2008.

Abstract

The roles of *Xylella fastidiosa* type I and type IV pili in movement and biofilm formation have been further defined. Type I pili appear to function in surface attachment whereas type IV are essential for motility and also play a role in secondary structure of biofilms. In addition, two regulatory systems involved in motility, disease and biofilm formation were identified. We provide the first evidence that twitching motility in *X. fastidiosa* is controlled by a signal transduction pathway (*pilG-chpC* cluster), which is highly similar to chemosensory systems controlling motility in several bacteria including *Pseudomonas aeruginosa*. We show that *pilL* is essential for twitching motility as three different insertional mutations in this gene resulted in a twitching-negative phenotype. We have also identified a *cheY* homolog, *chpY*, and show it is associated with twitching motility. We found that *chpY* is also involved in biofilm formation. Transmission electron microscopy revealed that type IV (and type I) pili are present in the *chpY*¯ mutant and its complement. In silico analysis of ChpY protein predicts its role in a chemosensory signal transduction relay and the presence of domains related to GGDEF and EAL suggests a possible role in biofilm formation. In addition we identified a gene near the *pilG-chpC* cluster, *tonB3*, that is also required for twitching. A second two component regulatory system (*pilR-pilS*) was also identified and its role in regulating type IV pilin production was assessed. We also report the production of monoclonal antibodies against *Xf* pili.

Introduction

Twitching motility (TM) functions in host colonization of many Gram-negative bacteria. *X. fastidiosa* (*Xf*) has both type I pili and type IV pili, and exhibits TM and biofilm formation (Meng et al, 2005). We have identified several genes in *Xf* associated with production of pili and phenotypes associated with mutants in the genes (ie. *fimA* (=*fimX*), *fimT*, *pilB*, *pilQ*, *pilR*, *pilX* and *pilY1*) (Li et al, 2007). In *P. aeruginosa* TM is regulated by a chemosensory system that involves two gene clusters *pilG-K* and *chpA-E*, that are analogous to bacterial chemotaxis systems that control swimming motility in response to environmental stimuli (Whitchurch et al, 2004). Chemosensory systems are composed of sensory receptors networking with components of cytoplasmic phosphorylation and dephosphorylation cascades. We describe three new genes in *Xf* that are associated with expression of TM. Two of them, *chpY* and *pilL*, are predicted to be part of a putative chemosensory system that controls TM in *Xf*; the third is *tonB3* that may be responsible for the transport of pili subunits outside of the cell. *ChpY* (ORF XP1757) was previously annotated as unnamed and part of a two-component regulatory system. In this report we predict by deduced protein structure that ChpY most likely functions as a phosphoacceptor. The *pilR-pilS* two component system is also known in other bacteria to be involved in type IV pili production and thus is key to regulation of twitching motility. We also report our progress on the production of monoclonal antibodies against *Xf* pili that we propose to use for development of diagnostic tests and eventually in the development of novel controls for Pierce's Disease.

Objectives

1. Characterize the putative type I pili gene cluster and phenotypes associated with genes.
2. Characterize two additional gene clusters (pilG-K/chpA-E and pilD-pilS) that are likely to be involved in regulation of type IV pili and related functions.
3. Development of monoclonal antibodies to *Xf*.

Activities Conducted to Accomplish Objectives:

1. Characterize the putative type I pili gene cluster and phenotypes associated with genes.
Characterization of *fimA*⁻ mutant phenotypes. *fimA* is the main gene in the Type I pili cluster. We developed a mutant for *fimA*, based on random mutation via transposon (*fimA*⁻). This mutation completely abolishes the production of type I pili, showing only type IV pili. Same results were shown by Feil et al. (2003) when mutants of *fimA* and *fimF* were obtained by a different methodology. We also generated a second mutant in this cluster *mrkD* (=*mrkD1* =XP0058), which gave the same phenotype (not shown). We expect that any other mutant in this cluster would exhibit similar phenotypes. It should be pointed that *fimA* (XP0063) is currently renamed as *fimX*. (XP0063 at *Xf* comparative genome project http://www.xylella.lncc.br/) but for this report we refer to as *fimA*.

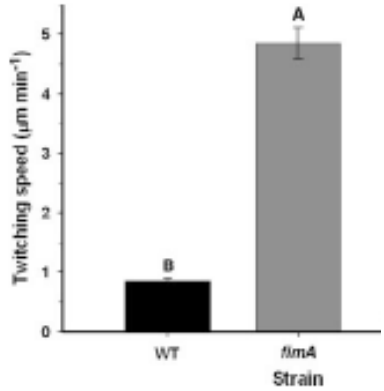


Fig. 1.1. Comparative migratory speeds of type IV pilus-mediated twitching movement of *Xf* wild-type (WT) cells and *fimA*- mutants. Letters indicate significant differences.

Motility and adhesion. We accessed the motility and adhesion force of *fimA*- in microfluidic chambers. We have shown that *fimA*- has increased motility (Fig. 1.1) and is less adherent to the microfluidic chamber surface under different flows than the wild-type (De La Fuent et al. 2007a).We hypothesize that the presence of the longer type IV pili somehow physically obstructs maximum contact of short pili with the substratum. Also, the type IV pili are dynamic constantly extending and retracting, keeping the cell in motion. This also possibly keeps wild-type cells from achieving maximum contact that is only achieved without the type IV pili (*pilB*⁻ mutant).

We also accessed the motility of *fimA*- on PW agar overlaid with cellophane using time-lapse microscopy. Twitching motility occurs as individual cells and as clusters of cells that move forward to the colony periphery. For complete movies see http://www.nysaes.cornell.edu/pp/faculty/hoch/movies/

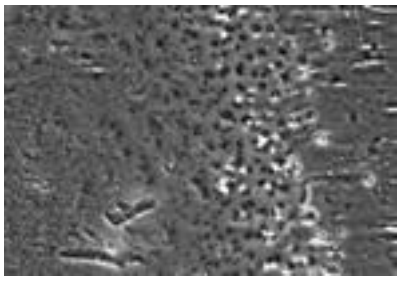


Fig. 1.2. Temporal and spatial observations of the colony fringes of *fimA*- mutant. Time lapses images were performed with an inverted IMT-2 Olympus microscope using a 20X phase-contrast objective and recorded with a CoolSNAP cf digital camera controlled by MetaMorph image software.

Biofilm formation and cell-cell aggregation. *fimA*⁻ mutant forms biofilms that have a more diffuse phenotype on surfaces (Fig. 1.2) as compared to the wild-type and to mutants that have only type IV pili (Li et al. 2007). Biofilms formed by the wild-type have a denser-appearing phenotype (Fig. 1.3) and therefore we hypothesize that type IV pili play an important role in biofilm secondary structure. Mutants that do not produce type I pili form biofilms that are sparse but appear to be made of dense clusters of cells, again suggesting a role for type IV pili in cell-cell attachment and thus secondary structure of biofilms.

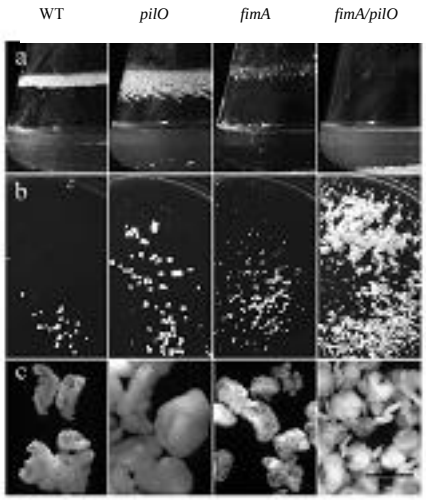


Fig. 1.3. Biofilm formation and cell-cell aggregation of *Xf* wild-type (Temecula) amd various pilus-mutants following seven days growth in culture. a) biofilm on inner surface of flasks; b and c) free-floating cell aggregates from the bottom of the flasks corresponds inversely to the amount of biofilm on flask sides. *pilO,* short type I pili only, *fimA*, longer type IV pili only and *fimA/pilO*, double mutant lacking both pili types (from Li et al. 2007).

Plant symptoms. Meng et al. (2005) has shown that *fimA*⁻ colonizes grapevine xylem more extensively than wild-type after 11 weeks. Recent *in planta* experiments (Burr & Hoch, 2006) showed that fimA- produced symptoms of PD earlier as compared to the wild-type under greenhouse conditions.

This research has provided further insight on the role of type I pili in the biology of *Xf* and in the development of Pierce's disease.

2A. Characterize a *Xf* gene cluster that carries orthologs found in bacterial chemosensory regulatory systems.
Identity of twitching mutants. Putative twitching-defective mutants were selected by the presence of a smooth colony margin (Li et al, 2007), i.e. the absence of a twitching-associated peripheral fringe. Analyses of genome sequence flanking the sites of transposon insertion revealed three mutants resulting from different insertions in ORF XP0874 (*pilL*). A second motility mutant resulted from an insertion in ORF XP1757 which resides 0.84 Mb downstream of the main chemosensory cluster (XP0871-XP0876). XP1757 is annotated as belonging to a two-component regulatory

system and was designated by us as *chpY* . The three (*pilL*$^-$), mutants and the *chpY*$^-$ mutant all exhibited a smooth colony margin on PW medium (Fig. 2.1B).

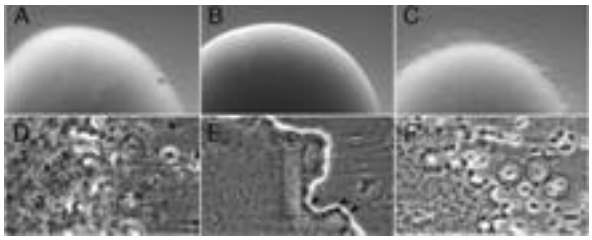


Fig. 2.1. Colony fringe phenotypes of the wild-type *X. fastidiosa* (A, D), *chpY*- mutant (B, E), and the complemented *chpY*$^-$ (C, F) grown on PW agar (A-C) and on cellophane overlaid on PW agar (D-F). Circled regions in D and F highlight rafts of aggregated cells moving away from the colony margins. Arrows in E denote denser regions of colony margin where possible rafts were prevented from moving outward from the colony. Boxed region of E shows individual bacteria that exhibited twitching motility. Figures E and F are frames taken from movies of *chpY*$^-$ mutant (E), and the complemented *chpY*$^-$ mutant (F), Scale bars, 0.5 mm (A-C) and 15 μm (D-F).

Sequence analysis of the *Xf* putative chemosensory cluster and related genes. Genes predicted to comprise a single *X. fastidiosa* chemosensory cluster involved in twitching motility are shown in Table 1. The *pilL*$^-$ mutants resulted from insertions in different regions of the ORF XP0874. This ORF encodes a multidomain protein that belongs to the family of CheA-like histidine kinases. *pilI*, predicted to encode a CheW-like protein that forms a ternary complex with PilL/ChpA and the methyl-accepting chemotaxis protein (MCP) PilJ; together functioning as an allosteric enzyme. Analysis of *pilJ* revealed it possesses characteristic domains found in MCPs that are required for formation of the ternary complex. A single copy of *pilJ* (XP0873) encoding a single 680 aa MCP is found in Temecula, and other strains of *X. fastidiosa* (data not shown). Upstream of *pilL* resides a *tonB* homolog XP0869. We named the ORF XP0869 *tonB3*, as it is the third *tonB* gene from the beginning of genome.

Table 1. Components of the chemosensory system of *X. fastidiosa* and related genes.

Gene ID[a]	Predicted protein	Synonym[b]	ORF Size (bp)[c]	Size (aa)[d]	MW[e] (kDa)	pI
XP0871	PilG	*CheY*	417	138	15	9.3
XP0872	PilI	CheW	531	176	19	6.1
XP0873	PilJ	MCP	2043	680	72.7	5.6
XP0874	PilL/ChpA	CheA	5178	1725	190	4.7
XP0875	ChpB	CheB	1170	389	41.4	4.5
XP0876	ChpC	CheW	471	156	17.1	4.6
XP1715	PilH	*CheY*	387	128	14	6.3
XP1757	ChpY	*CheY**	1701	566	63.7	5.1
XP0869	TonB3	TonB	900	300	33.2	10.0

[a] According to www.xylella.lncc.br; [b] Most similar protein or domain found in the chemosensory system of *E. coli*; [c] ORF length, in base pairs of nucleotides; [d] Protein length, in amino acids; [e] Molecular weight.*This protein has other non-Che related domains (see text for details).

To verify the insertional mutagenesis and to make sure the mutants had a single insertion, blotting and PCR were performed with the *pilL*, *chpY* and *tonB3* mutants (Fig. 2.2).

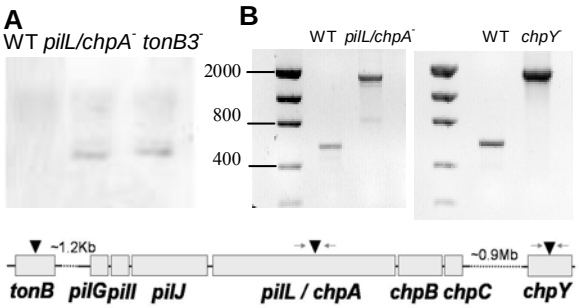
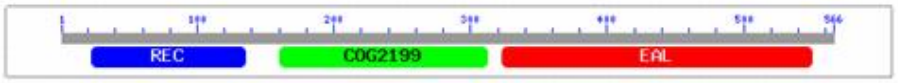


Figure 2.2. Confirmation of the insertional mutagenesis of *pilL/chpA*$^-$, *chpY*$^-$ and *tonB3*$^-$ mutants. (A). Blot showing the Tn5 insertion on *pilL/chpA*$^-$ and *tonB3*$^-$ mutants. (B) PCR showing the Tn5 insertion on *pilL/chpA*$^-$ and *chpY*$^-$.

Analysis of ChpY. The sequence analysis of ORF XP1757 predicts a protein belonging to the CheY family. It has a CheY-like receiver domain (REC) in its N-terminus that comprises amino acids 20 to 133 (Fig. 2.3). We have designated this protein as ChpY (chemosensory control of type IV pilus with CheY domain), which shows a high level of identity to orthologs in other *X. fastidiosa* strains. Based on sequence similarity with other response regulators, the conserved backbone β-α-loop was predicted (β2-α1-loop) for the CheY (REC) domain of ChpY. This putative 3D conformational structure supports its function as a response regulator associated with the chemosensory system of Xf. In addition to the CheY domain, ChpY also exhibits two other domains SDHSF and QVL that are related to GGDEF, and EAL domains. Proteins with GGDEF and EAL domains have been described as enzymes responsible for biosynthesis and degradation of c-di-GMP are shown to be involved in motility, aggregation, attachment, virulence, exopolysaccharide production and biofilm formation in many bacteria (Ryan et al. 2007).

Growth, biofilm, and pilus formation. Complementation analysis of the *chpY* mutant was accomplished by cloning the gene in pBBR1MCS-5 and transforming the mutant. No significant differences in growth rates between the mutant and complemented mutant were observed when compared to wild-type (Fig. 2.4). Therefore, the lack of twitching



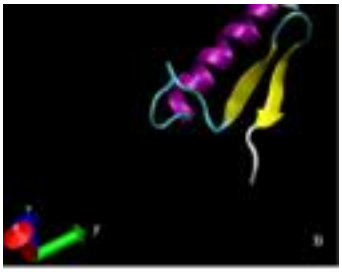


Figure 2.3. The ChpY predicted protein. The three distinct domains of ChpY are shown above and the ribbon structure of the three-dimensional model of the CheY domain of ChpY is shown to the right. Note the two beta-sheets and the alpha-helix typical of a functional REC domain

observed in mutants was not correlated with growth. The development of biofilms by wild-type, *pilL*⁻, *chpY*⁻ mutant and complemented *chpY*⁻ are shown in Fig. 2.5. The *chpY*⁻ mutant formed significantly more biofilm than the wild-type strain, and biofilm formed by complemented *chpY*⁻ was similar to the wild-type (Fig.). In contrast, the *pilL*- mutant formed less biofilm than the wild-type. Electron microscopy revealed that *pilL*⁻ and *chpY*⁻ mutants as well as the complemented *chpY*⁻ possess type I and type IV pili confirming that these genes are not involved in the pili biogenesis (Fig. 2.6). The twitching phenotype by *chpY*⁻ mutant therefore is due to the absence of a functional ChpY protein, which is predicted to interact with the type IV pilus motor and affect retraction. Similarly, we predict the abolishment of twitching in the *pilL*⁻ mutant is due to lack of histidine kinase binding to the MCP.

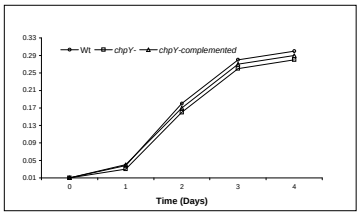


Fig. 2.4. Growth curve of *X. fastidiosa*. Growth curves for wild-type, the chpY⁻ mutant and the complemented mutant were compared. The growth curves were constructed from data obtained from 10-day period period (4-day shown) and performed as described by Galvani et al. (2007). Experiments were repeated at least three times using three replicates each.

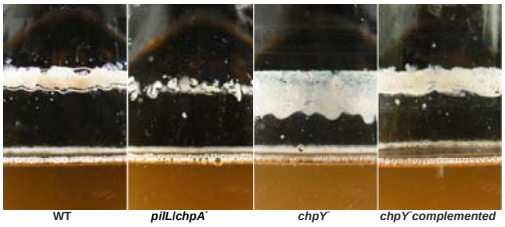


Fig. 2.5. Biofilm formation by *X. fastidiosa* grown in culture flasks following 10 days of growth with agitation. The *chpY*⁻ mutant biofilm layer was significantly wider than wild-type or *chpY*⁻ complemented biofilm. *pilL*⁻ biofilm was significantly reduced as compared to the wild-type.

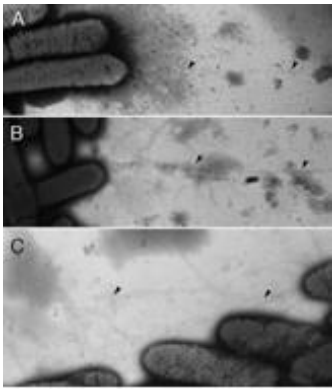


Fig. 2.6. Transmission electron micrographs of phosphotungstic acid negatively stained *Xf* cells of (A) *chpY*⁻ mutant, (B) complemented *chpY*⁻, and (C) *pilL*⁻ mutant. Arrowheads denote type IV pili. Circled regions denote presence of shorter type I pili. Scale bar, 1.0 µm.

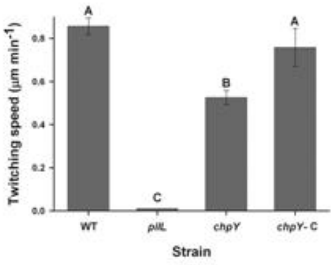


Figure 2.7. Speed of type IV pilus-mediated twitching movement of *X. fastidiosa* wild-type, *chpY*⁻ mutant, and complemented *chpY*⁻ mutant cells in microfluidic flow chambers. Values shown are means and standard errors from three independent experiments. Letters above bars indicate significant differences ($P = 0.02$).

Twitching motility. Examination of *pilL*⁻ and *chpY*⁻ mutants on cellophane overlaid on PW agar, and especially on agar surfaces alone, revealed colony morphologies with smooth margins consistent with loss of twitching function. Time-lapse video microscopy of cell movement on cellophane surfaces revealed twitching motility of the wild-type cells as well as in *chpY*⁻ mutant and *chpY*-complemented (Fig. 2.1). The *chpY*⁻ mutant, while having a smooth colony periphery, exhibited twitching motility within the confines of the colony.

Notably, the twitching cells did not break away from the colony periphery to form migrating rafts like wild-type or *chpY*-complemented cells. In contrast, all three *pilL*/*chpA*⁻ mutants were non-motile when observed in microfluidic chambers. The *chpY*⁻ mutant exhibited twitching movement against media flow, but at a lower rate than the wild-type and *chpY*-complemented. (Fig. 2.7). There was no difference in the speed of movement between cells of the *chpY*-complemented and wild-type isolate.

Twitching motility chemosensory model. We propose a model for TM chemosensory in *Xf* (Fig. 2.8).

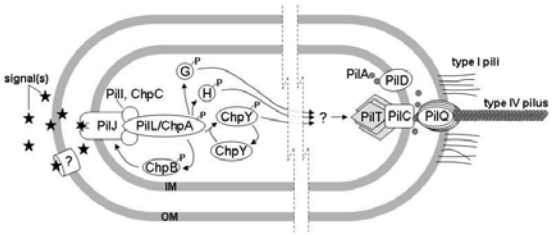


Fig. 2.8. Proposed model for chemosensory regulation of twitching motility in *X. fastidiosa*. PilJ, the single polar methyl-accepting chemotaxis protein, senses environmental signal(s).PilL/ChpA is a histidine kinase that phosphorylates the response regulators ChpY and PilG and the methylesterase ChpB. ChpC is an adaptor protein that couples PilL to PilJ. ChpB, is a methylesterase, mediating adaptation to a constant chemical concentration by adjusting the methylation level of the receptor. Phosphorylated ChpY and PilG modulate extension and retraction of type IV pili, thus driving motility.

Transcription analysis of the chemosensory cluster. We also investigated the effect of the transposon insertion in *pilL*/*chpA* on the transcription of neighboring coding

sequences by semi-quantitative RT-PCR. This analysis showed not only that wild-type expression levels are retained, but also that *pilG -chpC* comprise an operon (Fig. 2.9).

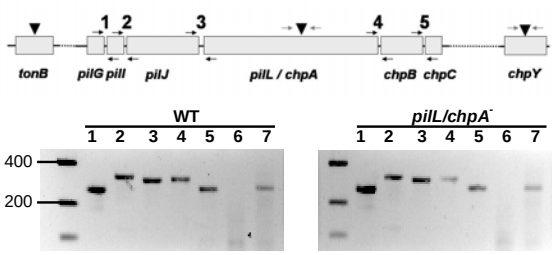


Fig. 2.9. RT-PCR showing operon structure of *pil chp* cluster. Total RNA treated with RNAse was used to amplify fragments indicated by black arrows in the top diagram. (1) *pilG-pilI*; (2) *pilI-pilJ*; (3) *pilJ-pilL*; (4) *pilL-chpB*; (5) *chpB-chpC*; (6) *pilG-pilI* with no reverse transcriptase but with DNA polymerase; (7) *pilG-pilI* fragment amplified from genomic DNA.

Construction a *pilL* null mutant strain of *X. fastidiosa* by allelic exchange. The construction of an allelic exchange mutant for *pilL*/*chpA* gene in *Xf* was performed according to Chatterjee et al. 2008. The disruption of the *pilL* locus in marker-exchange mutants was confirmed by sequencing and PCR. The *pilL* mutant strain was designated as *pilL*pLC2. The achievement of the allelic exchange mutagenesis in a particular large gene opens a new perspective to mutagenesis of new genes of interest in *X. fastidiosa* by our group. We are beginning to characterize the *pilL* pLC2 mutant and anticipate its similarity to previous *pilL* mutants in terms of motility and biofilm formation.

Differential expression of *pilL* under different nutrition status. Nutritional status of media including certain amino acids has been implicated as environmental stimuli to chemosensory systems (Whitchurch et al., 2004). We used semiquantitative RT-PCR to investigate the signals that might be involved in triggering the chemosensory cluster of *Xf*. We have found that differential expression is observed in the absence of soytone (Fig. 2.10). It should be noted that the growth of *Xf* is also reduced in the absence of soytone but the cells are still able to express other genes such as *fimA*.

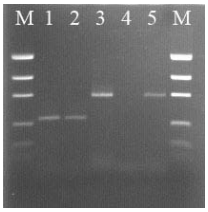


Fig. 2.10. Semiquantitative RT-PCR for *pilL* expression of *X. fastidiosa* wild-type. Lane M Invitrogen low DNA Mass Ladder: 2Kb; 1.2Kb; 0.8Kb; 0.4Kb; 0.2Kb; 0.1Kb. Lane 1 (control) *fimA* expression from cells growing on regular PW. Lane 2 (control) *fimA* expression from cells growing on PW in absence of soytone. Lane 3 *pilL* expression from cells growing on regular PW. Lane 4 *pilL* expression from cells growing on PW in absence of soytone. Lane 5 *pilL/chpA* expression from cells growing on PW in absence of dipotassium hydrogen phosphate.

Other experiments analyzing the influence of all of the 20 different amino acids in this system have not added new information. In our current grant we will study more deeply the possible *Xf* environmental clues using *E.coli* mutants. We hope to find information that can be useful to control *Xf*.

2B. Characterize the regulatory system *pilS-pilR* that is likely to be important for twitching motility in *Xylella fastidiosa*. The regulatory system *pilS-pilR* is part of the cluster *pilD-pilS*. This system is responsible to control the expression of *pilA1* (the main type IV pilin unit) in *P. aeruginosa* and *M. xanthus* (Boyd, 2000, Wu and Kaiser, 1997). In order to understand the regulation of *pilA1* pilin, we used semiquantitative RT-PCR to investigate whether the pair *pilS-pilR* is responsible for *pilA* expression (Fig. 2.11 and 2.12).

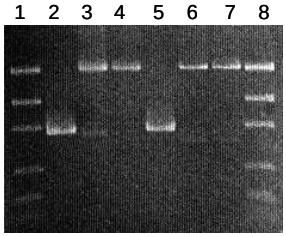


Fig. 2.11. Presence of *pilR* gene in *Xf*. Wild-Type and pilR- mutants Lanes 1,8 Marker. Lanes 2,5. *pilR* in wild-type . Lanes 3 ,6 *pilR* in *pilR*- mutant 1. Lanes 4,7 *pilR* in *pilR*- mutant 2 (note: that the size of the fragment is showing the insertional mutation on *pilR*)

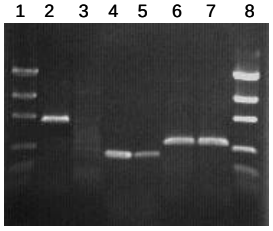
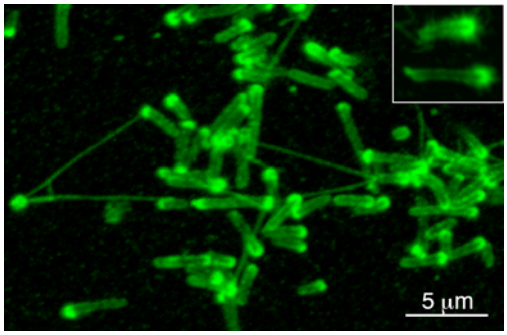


Fig.. 2.12. Semiquantitative RT-PCR for *pilA* and *pilR* expression on *X. f* wild-type and *pilR*- mutant. Lanes 1,8 Marker. Lane 2 *pilR* expression on WT. Lane 3 *pilR* expression on *pilR*- mutant. Lane 4 *pilA* expression on WT. Lane 5 *pilA* expression on WT *pilR*- mutant. Lane 6 (control) *fimA* expression on WT. Lane 7 *fimA* expression on WT *pilR*- mutant.

The semiquantitative RT-PCR for *pilA1* expression on a *Xf pilR*- mutant showed a slight difference in the expression of *pilA*. Given that *pilA* has multiple copies in the genome, further studies to understand the role of each are necessary. Our results suggest that *pilS-pilR* system is able to control the activity of *pilA* at some level. Further studies on regulation of type IV pili on *Xf* will be continued by our group due to its importance as a target to control.

Production of MAb's against *Xf*. Monoclonal antibodies (MAb's) will be used for further characterization of *Xf* pili and other surface characteristics important to further understand the biology of the pathogen. In addition, they may be useful in inhibition of migration and colonization of *Xf* in vitro and in planta, and in development of a field-applicable diagnostic bio-detection sensor with enhanced sensitivity and speed of detection. We proceeded to produce MAb's toward various surface proteins of *Xf*. Mice were immunized with whole live cells of wild-type *Xf*. Blood serum from immunized mice exhibited excellent recognition of *Xf* cell surface antigens including both type I and type IV pili (Fig. 3.1). Initial MAb production by hybridoma cells was similar toward many of the desired *Xf* antigens; however, with time the hybridoma cell lines producing MAbs toward pili either ceased production or could not be maintained in

culture. Nevertheless, several potentially useful MAb producing hybridoma cell lines were obtained (Fig. 3.2) and are being further studied.



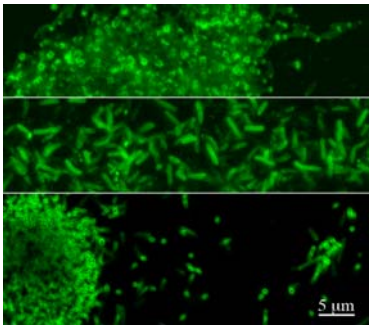


Fig. 3.1. (far left) Immunocytochemical staining of WT *Xf* with mouse serum. Long type IV pili and shorter type I pili are associated with one cell pole.

Fig. 3.2. Examples of three different MAbs staining predominately cell pole (upper), and outer cell body (middle, lower).

Conclusions

We characterized *fimA*, the most important gene for type I pili. We have shown the importance of type I pili in biofilm formation, cell-cell aggregation, movement and migration *in vitro* and *in planta*. We provide evidence that *Xf pilL* is part of a complex chemosensory system that controls type IV pilus-mediated motility in *Xf*. We were able to prove that the chemosensory cluster has an operon organization and that the production of type IV pili is not affected by mutations in this cluster. The successful construction of an allelic mutant for *pilL* will increase the knowledge about this gene and open perspectives for new genes involved in virulence to be targeted. We also characterized *chpY*, a former hypothetical gene that now is known to play a role in TM and biofilm formation in *Xf* .We hypothesize that *chpY* might also play a role in the TM chemosensory system. Complementation of this gene restored wild-type motility and biofilm formation levels, proving its important roles. Further research is required to understand the role of *tonB3* in TM. As our knowledge of *Xf* type IV pili production, regulation and functioning advances, the coordinate regulation of them as a virulence factor is being elucidated. Understanding how this regulation occurs is central to determining how to counter movement and plant colonization. The targeting of genes required for pathogenicity including colonization may be an effective strategy. The examination of the role of other genes from the *pilG-chpC* cluster and their relation to TM is a crucial step in this process. Another allelic mutant for the cluster is under construction: *pilJ* the important MCP we hope to get insights on the environmental clues that drive the chemosensory system and the movement of *Xf* inside plants. The production of monoclonal antibodies against *Xf* pili will certainly help contribute to this step as well as to the development of diagnostic tools and eventually for the development of novel controls for Pierce's Disease.

Publications resulting from the project

Li, Y., G. Hao, , C. D. Galvani, Y. Meng, L. De La Fuente, H. C. Hoch, and T. J. Burr. 2007. Type I and type IV pili of *Xylella fastidiosa* affect twitching motility, biofilm formation and cell-cell aggregation. Microbiology 153: 719–726.

De La Fuente, L., T. J. Burr, and H. C. Hoch. 2007. Mutations in Type I andType IV Pilus Biosynthetic Genes Affect Twitching Motility Rates in *Xylella fastidiosa*. J Bacteriol 189: 7507–7510.5

Galvani, C. D, Y. Li, T. J. Burr, and H. C. Hoch. 2007. Twitching motilityamong pathogenic *Xylella fastidiosa* isolates and the influence of bovine serum albumin on twitching-dependent colony fringe morphology. FEMS Microbiol Lett. 268: 202-208.

De La Fuente, L., E. Montanes, Y. Meng, Y. Li, T. J. Burr, H. C. Hoch, and M. Wu. 2007. Assessing adhesion forces of type I and type IV pili of *Xylella fastidiosa* using a microfluidic flow chamber. Appl. Environ. Microbiol. 73:2690–2696.

Presentations on research

Burr, T. J., H. C. Hoch, L. Cursino, Y. Li, L. De La Fuente and C. Galvani. 2006. The roles that different pili classes in *Xylella fastidiosa* play in colonization of grapevines and Pierce's disease Proceedings of the Pierce's Disease Research Symposium 2006, p. 124-126.

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De La Fuente, L., Zaini, P. A., Cursino, L, Lin, H., Burr, T., Hoch, H. 2008. Environmental factors affecting twitching motility, biofilm development, and aggregation by *Xylella fastidiosa* Phytopathology 98: (6) S45-S45.

Research relevance statement
We aim to provide an improved understanding of how different pili types on *X. fastidiosa* affect on movement, biofilm formation, aggregation and development of symptoms inside of grapevine. This information will provide insight on how the bacterium colonizes grapevines and will be important for the development of PD controls.

Lay summary of the results
X. fastidiosa was found to possess two types of appendages called pili (type I and type IV) that play important roles in motility, disease and biofilm formation. Movement is essential for the bacterium to colonize grapevines. We determine that the bacterium can move against the transpiration stream. Biofilms, which are organized cell clusters, serve to plug the grape vascular system and also to protect the bacterium from environmental stresses.

Our research demonstrated that both pili types play roles in biofilm formation. We show that the longer type IV pili are essential for movement and also enhance cell-cell aggregation and thus the secondary structure of biofilms. Type I pili, which are shorter, are important for attachment of cells to a surface but do not function in motility.

We have identified two regulatory systems that are important for motility and biofilm formation. The *Xf* genome carries one copy of a predicted chemosensory regulatory gene cluster. A mutation in a key gene of the cluster, *pilL*, resulted in a motility-minus phenotype. In addition, two other genes associated with the chemosensory cluster and involved in motility were identified. Our goal is to identify environmental signals that trigger the chemosensory regulation and subsequently block the ability of the bacterium to move and colonize grapevines.

Another regulatory system was also identified that is believed to regulate the production of the major protein, pilin, that makes up type IV pili. Again, strategies for interfering with this regulation are targets we are pursuing as a means of disease control.

Significant progress has been made in the identification of monoclonal antibodies that will be used to study pathogen biology and for development of diagnostic tools for *Xf*.

Funding has been expended and remaining funds returned to the grants program.

Summary and status of intellectual property produced during this research project
None to date

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