EXPLOITING A CHEMOSENSORY SIGNAL TRANSDUCTION SYSTEM THAT CONTROLS TWITCHING MOTILITY AND VIRULENCE IN *XYLELLA FASTIDIOSA*

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ABSTRACT

Previously we demonstrated that twitching motility in *Xylella fastidiosa* is dependent on an operon encoding signal transduction pathway proteins (*pilG, pilI, pilJ, pilL, chpB* and *chpC*), which is related to the system that controls flagella movement in *Escherichia coli*. We report three advances in examining this system. First, we have examined the operon genes more closely. We have discovered that the operon is essential for the twitching phenotype, determined the importance of each gene individually, and discovered that the *chpB* gene is non-functional. Further characterization of these genes is underway. Second, we have found that the chemoreceptor, PilJ, is localized to one pole of the cell. We are currently determining if the chemoreceptors and pili are found at the same pole of the cell. Third, we have continued our examination of *chpY*, a gene similar to *pilG*, which plays a role in Pierce's disease development.

LAYPERSON SUMMARY

This project involves studying the chemical sensing pathway by which the plant pathogen *Xylella fastidiosa* is able to control its movement within the plant environment. We examined a gene cluster essential for cell movement (twitching motility), we identified where the initial protein regulating the signaling response is located in the cell, and we determined that chemical sensing is important for developing disease symptoms. These results give insight into targets for preventing Pierce's disease.

INTRODUCTION

Bacteria sense and respond to changes in their environment, integrating the signals to produce a directed response. *Xylella fastidiosa* (*Xf*) is a non-flagellated, xylem-restricted Gram-negative bacterium that moves within grapevines via twitching motility that employs type I and type IV pili (Meng et al. 2005). Movement appears to be controlled by a chemosensory system similar to that first reported in *E. coli* in which a group of *che* genes regulates the rotational movement of flagella. Transmembrane chemoreceptors bind chemical stimuli in the periplasmic domain and activate a signaling cascade in their cytoplasmic portion to ultimately control the direction of flagella rotation (Hazelbauer et al. 2008). We previously found that the chemosensing gene cluster is an operon (named Pil-Chp) that regulates type IV pili, and that disruption of the operon leads to a decrease in Pierce's disease (PD) symptoms (**Figure 1**). Herein, we further characterize the genes in the Pil-Chp operon and describe our advances in understanding the role of *pilJ* signaling in *Xf*.

OBJECTIVES

1. Complete characterization of the single chemosensory regulatory system of *Xf* and its function in PD. In particular, we will focus on its role in mediating bacterial movement and biofilm formation. Toward this end we will:
   a. Obtain *Xf* mutants in the *pilJ* gene that encodes the single methyl-accepting chemotaxis protein in *Xf*.
   b. Assess virulence and motility of *pilJ* mutants in grapevines, as well as previously created mutants deficient in related chemosensory genes, *pilL* and *chpY*.
2. Identify environmental signals that bind PilJ and activate chemosensory regulation. Toward this end we will:
   a. Express PilJ or a chimeric form of PilJ in a strain of *E. coli* previously deleted of all methyl-accepting chemotaxis protein genes.
   b. Subsequently, candidate signals will be screened using the above *E. coli* system for activation of motility.

RESULTS AND DISCUSSION

Construction of the *Xf* chemosensory operon null mutant strains. The construction of a non-polar, allelic exchange mutant of *pilJ* gene in *Xf* was performed according to Chatterjee *et al*. 2008 with slight modifications. We also disrupted the other genes in the Pil-Chp operon: *pilG*, *pilI*, *chpB*, and *chpC*. The disruption of each gene in marker-exchange mutants was confirmed by PCR (not shown). The *pilL* gene was disrupted previously with polar transposon mutations (Hoch et al. 2008a).

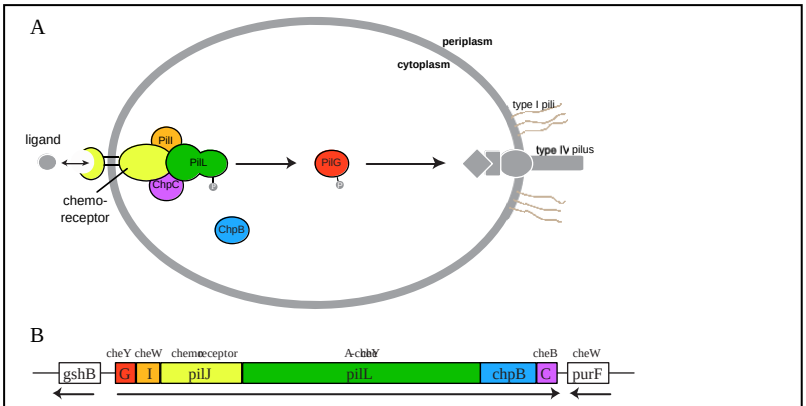


Figure 1. **The *Xf* chemotaxis system.** A) Model for chemosensory regulation of twitching motility in *Xf*. The chemoreceptor PilJ senses environmental signal(s). ChpC/PilI couples PilL to PilJ. PilL phosphorylates its hybrid CheY-like receiver domain and PilG. ChpB is homologous to ligand adaptation proteins but appears non-functional. B) The Pil-Chp chemosensory operon genes with *E. coli* homologous genes shown above and direction of transcription shown below.

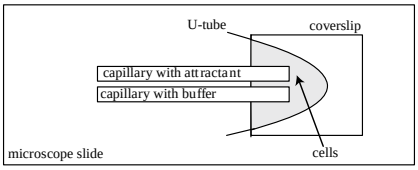
Construction of plasmids to complement the *Xf* chemosensory operon null mutant strains. To complement the non-polar *pilJ, pilG, pilI, chpB,* and *chpC* gene disruptions, we needed to construct plasmids containing the chemotaxis operon promoter region. The Pil-Chp operon lies 256 bp downstream of the unrelated gene *gshB*, which is transcribed in the opposite direction (**Figure 1**). Based on computer programs and the spacing between *gshB* and the Pil-Chp operon, we cloned two potential Pil-Chp operon promoters into *Xf*-compatible plasmids. We then cloned the various Pil-Chp genes into these constructs. We are currently preparing to transform the constructs into the null mutants in order to perform complementation studies.

Twitching motility of the *Xf* chemosensory operon null mutant strains. Previously we observed that the *pilL* mutant was twitching minus on both PW agar surfaces and in microfluidic chambers (Hoch et al. 2008a). Examination of the *pilJ* mutant on PW agar surfaces revealed colony morphologies with smooth margins consistent with loss of type IV pili twitching motility function (data not shown) (Meng et al. 2005). These findings were confirmed by measuring twitching motility of cells in microfluidic chambers. Similarly, the *pilG* and *pilI* mutants exhibited no movement, whereas the *chpB* mutant performed twitching motility like wild-type cells. We are currently characterizing the *chpC* mutant.

Identifying the chemosensory attractant. To examine what sap component results in a motility response, the putative *Xf* chemoreceptor was expressed in *E. coli*. The *pilJ* gene failed to complement the *E. coli* chemotaxis system (Hoch et al. 2009). To facilitate PilJ functioning in an *E. coli* system, we constructed chimeric chemoreceptors that contained the periplasmic ligand binding domain of the *Xf* PilJ fused to the cytoplasmic signaling domain of the *E. coli* serine chemoreceptor, Tsr. The chimera successfully activated the *E. coli* CheA kinase as the CheA binding site is maintained in the Tsr cytoplasmic portion (Hoch et al. 2009).

To confirm that PilJ binds to a molecule in grape sap, we tested the chimeras using a modified capillary assay (Grimm and Harwood, 1997). In this assay, a chamber is created between a microscope slide and coverslip separated by a capillary tube bent into a U-shape (**Figure 2**). Capillaries containing potential attractant or buffer in 2% agar are placed in the chamber. Cells suspended in aerated buffer are then added. Cells expressing the ligand adaptation system "sense" ligand via the chemoreceptors and migrate to the tip of the attractant-containing capillary creating a visible dense swarm of cells within 10-15 minutes. Cells expressing the chimera construct failed to respond to saps known to support twitching motility of *Xf*. Numerous reasons could explain this result. The attractant may have been at a concentration too low for assay detection, the attractant may require a *Xf* transporter protein to enter the periplasmic space, or the attractant may bind a *Xf* periplasmic binding protein before docking to the chemoreceptor. Alternative approaches are being explored.

A



B

	wt E. coli	chimera
LB media	+	nd
10mM serine	+	nd
PW media	nd	-
V. riparia	nd	-
V. viniferia	nd	-

Figure 2. Modified capillary assay and results. A) Cells are placed in a chamber with capillaries filled with agar and the appropriate attractant. If a capillary contains an appropriate chemical stimulus, the cells will swarm around the capillary tip. B) Cells were grown in T broth at 30°C and induced at log phase. At OD_{600}=0.4 they were washed 3 times in 10mM phosphate buffer pH 7.0 with 0.1mM EDTA and resuspended to OD_{600}=0.01. Capillary tubes were filled with 2% agar and appropriate attractant (LB media or serine for wt cells; PW media or sap for chimera-expressing cells) or buffer. Cells either swarmed (+) or did not swarm (-) at the tips as observed by 40X magnification. nd = not determined.

Localization of the chemoreceptor. Chemoreceptor localization has been studied in only a handful of organisms and found to be polar or cytoplasmic (Maddock and Shapiro 1993; Harrison et al. 1999; Bardy and Maddock 2005; DeLange et al. 2007). In *E. coli*, chemoreceptors cluster into a polar lattice that presumably allows the receptors to work in concert and amplify the signal (Parkinson et al. 2005). As a result, the chemoreceptors are physically at a distance from the flagella. In *Pseudomonas aeruginosa* the chemoreceptor PilJ is polar localized, however it was found to be at both poles and its location relative to the type IV pili was not identified (DeLange et al. 2007). We wish to learn if the *Xf* PilJ is a) polar localized, b) at one or both poles, and c) if it co-localizes with the pili. To answer this question, we expressed PilJ protein for antibody production and used it to label the chemoreceptor. We discovered that PilJ is expressed at a single cell pole (**Figure 3**). Immunocytochemical localization of an antibody that highlights an array of *Xf* proteins, including pili, revealed a distinct labeling at one end of the *Xf* cells consistent with the single pole location of type I and type IV pili (Meng et al. 2005; Hoch et al 2008b). We will next perform co-localization experiments to determine if the chemoreceptors and pili are at the same or opposite pole.

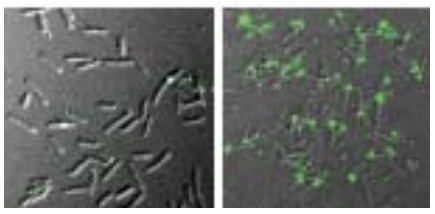


Figure 3. Localization of PilJ. Microscope images of wt *Xf* cells with anti-PilJ antibody (left) or anti-*Xf* antibody (right) that highlights the pili.

Pil-Chp operon and ChpY. The *chpY* gene lies downstream of the Pil-Chp operon and has homology to the *Xf pilG* gene. In a similarly organized chemosensing-like system, the *P. aeruginosa* Pil-Chp operon has downstream genes that produce proteins that associate with the operon protein products (Whitchurch et al. 2004). In addition to the *pilG*-like domain, the *chpY* has GGDEF and EAL related regions indicating its involvement in biofilm formation (Burr et al 2008). Deletion of *chpY* results in reduced cellular motility, unaltered pili biogenesis, and increased biofilm formation (Burr et al 2008).
To determine if there was a regulatory relationship between the Pil-Chp operon and *chpY*, we performed RT-PCR on the pill gene in a *chpY* null strain. When *chpY* is deleted, *Xf* shows no changes in the expression of *pilL* (**Figure 4a**). However, *chpY*

does have an effect on PD. Grapevines inoculated with the *chpY* mutant had increased PD progression compared to a wt *Xf* infection (**Figure 4b**), which may stem from increased biofilm formation or reduced twitching motility.

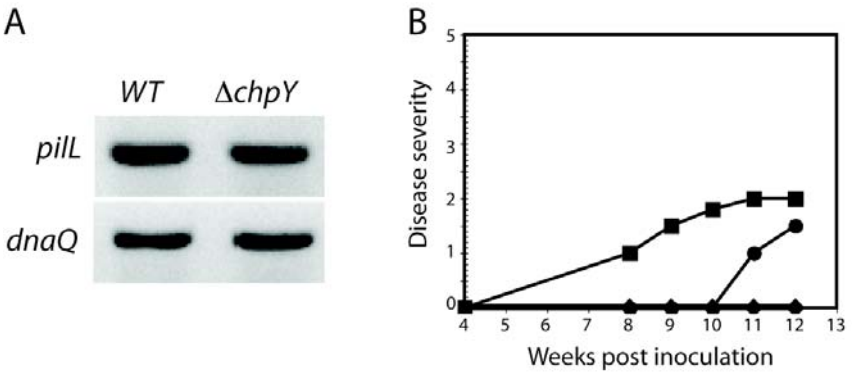


Figure 4. **Pil-Chp operon and the importance of ChpY for Pierce's disease.** A) The expression of the *pilL* gene from the Pil-Chp operon is unaffected by the presence of *chpY*. B) Grapevines (n=6) inoculated with wt *Xf* (circle), *chpY* mutant cells (square), or buffer (triangle) were assessed for disease progression over 12 weeks.

CONCLUSIONS

Our results with the Pil-Chp mutants show that the operon is required for twitching motility in *Xf*. Interestingly, some of the genes in the operon may not be functional. Currently we are studying additional phenotypes of the mutants including growth and biofilm formation. We have determined that PilJ is polar localized in *Xf* and will learn if it is co-localized with the pili. We are attempting new approaches to find the chemosensing stimuli in grape sap. Additionally, we are exploring *chpY*, which does not appear to be directly involved in chemosensing, but plays a role in biofilm formation and twitching motility.

REFERENCES CITED

Bardy, S.L., and Maddock, J.R. 2005. Polar localization of a soluble methyl-accepting protein of *Pseudmonas aeruginosa*. *J. Baceriol*. 187:7840-7844.

Burr, T.J., Hoch, H.C., Lindow, S., De La Fuente, L., Cursino L, and Zanini, P.A. 2008. The roles that different pili classes in *Xylella fastidiosa* play in colonization of grapevines and Pierce's disease pathogenesis. In *Pierce's Disease Research Symp. Proc.*, pp. 93-95. San Diego: Calif. Dep. Food Agric.

Chatterjee, S., Wistrom, C., and Lindow, S.E. 2008. A cell-cell signaling sensor is required for virulence and insect transmission of *Xylella fastidiosa*. *Proc. Natl. Acad. Sci. USA* 103:4198-4203.

DeLange, P.A., Collina, T.L., Pierce, G.E., and Robinson, J.B. 2007. PilJ localizes to cell poles and is required for type IV pilus extension in *Pseudomonas aeruginosa*. *Curr. Microbiol*. 55:389-395.

Grimm, A.C., and Harwood, C.S. 1997. Chemotaxis of *Pseudomonas* spp to the polyaromatic hydrocarbon napthtalene. *Appl. Environ. Microbiol.* 68:5789-5795.

Harrison, D.M., Skidmore, J., Armitage, J.P., and Maddock, J.R. 1999. Localization and environmental regulation of MCP-like proteins in *Rhodobacter sphaerodies*. *Mol. Microbiol*. 31:885-892.

Hazelbauer, G.L., Falke, J.J., and Parkinson, J.S. 2008. Bacterial chemoreceptors: high-performance signaling in networked arrays. *Trends Biochem. Sci*., 33:9-19.

Hoch H.C., Burr T.J., Mowery, P., Cursino L., Zaini P., and De la Fuente, L. 2008a. Exploiting a chemosensory signal transduction system that control twitching motility and virulence in *Xylella fastidiosa*. In *Pierce's Disease Research Symp. Proc.*, pp. 110-113. San Diego: Calif. Dep. Food Agric.

Hoch H.C., and Burr, T.J. 2008b. Understanding control of *Xylella fastidiosa* cell aggregation: importace in colonization and biofilm development in grapevine and shartpshooter foregut. In *Pierce's Disease Research Symp. Proc.*, pp. 106-109. San Diego: Calif. Dep. Food Agric.

Hoch H.C., Burr T.J., Mowery, P., Cursino, L., Zaini, P., and De la Fuente, L. 2009. Exploiting a chemosensory signal transduction system that control twitching motility and virulence in *Xylella fastidiosa*. In *Pierce's Disease Research Symp. Proc.*, pp. 92-96. San Diego: Calif. Dep. Food Agric.

Maddock, J.R., and Shapiro, L. 1993. Polar location of the chemoreceptor complex *in Escherichia coli* cell. *Science*. 259:1717-1723.

Meng, Y., Galvani, C.D., Hao, G., Turner, J.N., Burr, T.J., and Hoch, H.C. 2005. Upstream migration of *Xylella fastidiosa* via pilus-driven twitching motility. *J Bacteriol*. 187: 5560–5567.

Parkinson, J.S., Ames, P., and Studdert, C.A. 2005. Collaborative signaling by bacterial chemoreceptors. *Curr. Opin. Microbiol*. 8:1-6.

Whitchurch, C.B., Leech, A.J., Young, M.D., Kennedy, D., Sargent, J.L., Bertrand, J.J., Semmler, A.B., Mellick, A.S., Martin, P.R., Alm, R., Hobbs, M., Beatson, S.A., Huang, B., Nguyen, L., Commolli, J.C., Engel, J.N., Darzins, A., and Mattick, J.S. 2004. Characterization of a complex chemosensory signal transduction system which controls twitching motility in *Pseudomonas aeruginosa*. *Mol. Microbiol*. 52:873-893.

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