Antonie van Leeuwenhoek (2005) 87:253–257
DOI 10.1007/s10482-004-4620-x

Short Communication

Transcription analysis of *pil*S and *xps*EL genes from *Xylella fastidiosa*

Patricia P. Coltri* and Yoko B. Rosato
*Centro de Biologia Molecular e Engenharia Genética (CBMEG), Universidade Estadual de Campinas, P.O. Box 6010, CEP 13087-930, Barão Geraldo, Campinas, SP, Brazil; *Author for correspondence: Present address: Laboratório Nacional de Luz Síncrotron (LNLS), P.O. Box 6192, CEP 13084-971, Campinas, São Paulo, Brazil (email: ppcol@unicamp.br)*

Received 26 July 2004; accepted in revised form 12 October 2004

*Key words: pil*S, Transcription, *xps*, *Xylella fastidiosa*

Abstract

Xylella fastidiosa is a xylem-limited phytopathogen responsible for diseases in several plants such as citrus and coffee. Analysis of the bacterial genome revealed some putative pathogenicity-related genes that could help to elucidate the molecular mechanisms of plant–pathogen interactions. In the present work, the transcription of three genes of the bacterium, grown in defined and rich media and also in media containing host plant extracts (sweet orange, 'ponkan' and coffee) was analyzed by RT-PCR. The *pilS* gene, which encodes a sensor histidine kinase responsible for the biosynthesis of fimbriae, was transcribed when the bacterium was grown in more complex media such as PW and in medium containing plant extracts. The *xps* genes (*xpsL* and *xpsE*) which are related to the type II secretion system were also detected when the bacterium was grown in rich media and media with 'ponkan' and coffee extracts. It was thus observed that *pilS* and *xpsEL* genes of *X. fastidiosa* can be modulated by environmental factors and their expression is dependent on the nutritional status of the growth medium.

Introduction

Xylella fastidiosa (Wells et al. 1987) is a xylem-limited bacterium causing several diseases in tropical and sub-tropical regions. The bacterium induces symptoms in several plants such as plum, peach, almond, oleander and grapevine and is responsible for serious economic losses (for a review, see Hopkins and Purcell 2002). In Brazil, the major impact of this pathogen was in sweet orange (Rossetti et al. 1990) and coffee plants (Beretta et al. 1996). Currently it is assumed that the bacterium blocks the xylem vessel of infected plants either by secreting some exopolysaccharide or by mechanical occlusion leading to hydric stress and to the appearance of the symptoms (Mircetich et al. 1976; Hopkins 1989). However, the specific molecular mechanisms of interaction between the plant and the pathogen are not clearly understood. Interestingly no known pathogenicity-related genes such as *avr* or *hrp* have been found in the genome of *X. fastidiosa* (Simpson et al. 2000) indicating that other unsuspected factors might be involved in the elicitation of the symptoms as well as in the interaction of the pathogen with its hosts (Dow and Daniels 2000; Bhattacharyya et al. 2002; Costa de Oliveira et al. 2002; Nunes et al. 2003).

Among the potential candidates involved with pathogenicity are genes of the *rpf* system, *gum* operon, fimbriae biosynthesis and signal transduction systems (Dow and Daniels 2000; Machado et al. 2001). The fimbriae are important in the

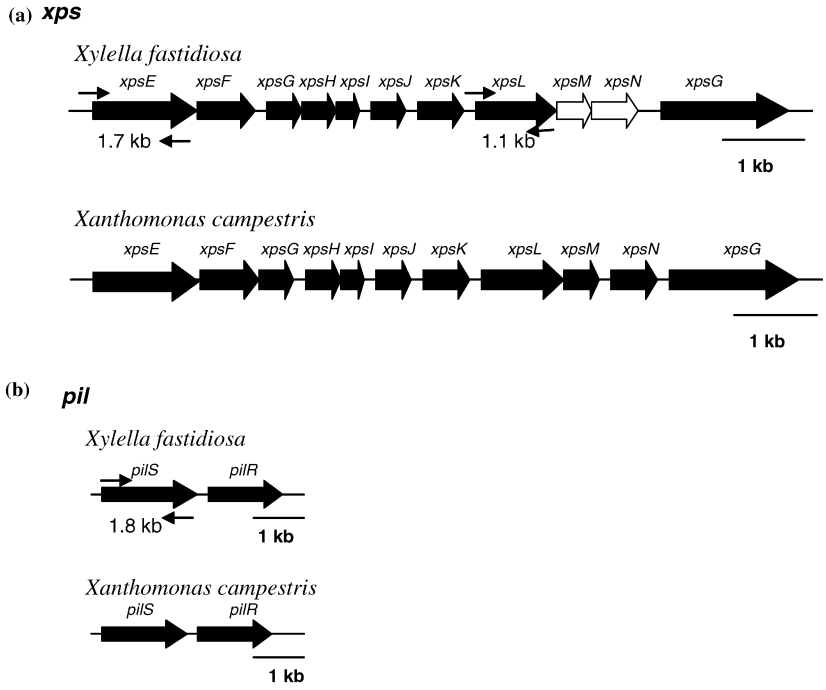

Figure 1. Genetic organization of the *X. fastidiosa* (a) *xps* operon and (b) *pil*S locus compared to the region of homologous genes in *X. campestris* pv. *campestris*. The black bar represents the DNA sequence and the overlying arrows represent the genes. The 'white' arrows in *X. fastidiosa xps* operon represent hypothetical genes with homology to corresponding genes in *X. campestris*. The thin arrows represent the specific primers designed to amplify *xpsE*, *xpsL* and *pilS X. fastidiosa* genes and sizes of the predicted products are indicated.

adherence of bacteria with cells and with the host plant vessels. Cellular adhesion is probably mainly responsible for blocking the xylem and the passage of water and nutrients to the upper parts of the plant, thus leading to hydric stress and contributing to the appearance of symptoms. Twenty-six genes related to the function and biosynthesis of fimbriae were found in the *X. fastidiosa* genome including a sensor/effector system, composed by *pilS* and *pilR*. Signal transduction systems have been considered important in the adaptation to different environmental conditions (Parkinson 1993). The type II secretion system, represented by the *xps* system, is commonly found in Gram-negative pathogens such as *Xanthomonas*, *Klebsiella* and *Erwinia* (Salmond 1994). It is responsible for secretion of extracellular enzymes such as cellulases and polygalacturonases in a Sec-dependent manner (Russel 1998; Lee et al. 2001), and it has also been related to virulence (Salmond 1994).

In this work, the transcription profiles of three genes from *X. fastidiosa* were analyzed by RT-PCR: *pilS* (1.8 kb) encodes a sensor protein involved in fimbriae biosynthesis whereas *xpsL* (1.1 kb) and *xpsE* (1.7 kb) are part of a large cluster of genes homologous to an operon found in *Xanthomonas campestris* involved in secretion of extracellular enzymes (Figure 1). The transcription of these three genes was analyzed in *X. fastidiosa* grown under different cultural conditions (complex, defined and amended with plant extracts media).

X. fastidiosa 9a5c was routinely grown in PW medium (Davis et al. 1981) at 28 °C. The inoculum was prepared by growing the bacterium in PW medium for 4 days. The bacterium was then transferred to XDM* medium and allowed to grow for another 4 days. XDM* medium contained 0.23 g KH_2PO_4, 0.10 g $MgSO_4$. $7H_2O$, 1.1 g glutamic acid, 4 ml glycerol and 0.5 g ferric pyrophosphate per liter and was the first medium to be described in a meeting as a minimal medium by Lemos prior to the publication of the XDM medium (Lemos et al. 2003). The cells were then collected and transferred to XDM* enriched with plant extracts (1% w/v) prepared from different plant hosts (*Citrus sinensis*, *Citrus reticulata* cv Ponkan and *Coffea arabica*) as

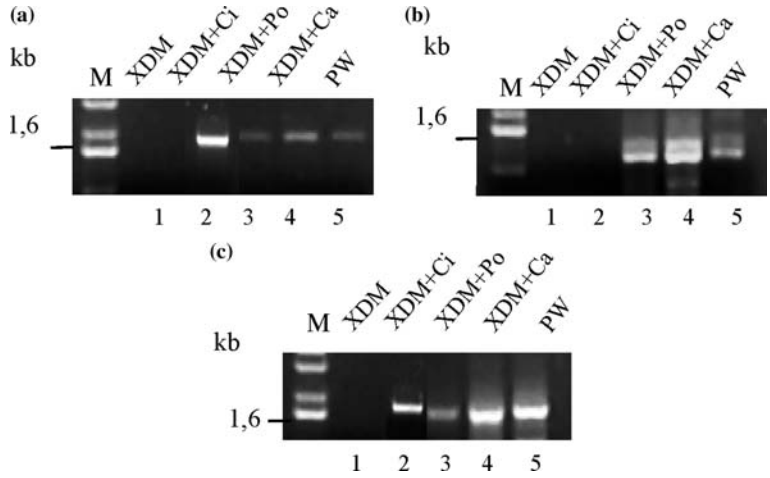

Figure 2. RT-PCR products of *X. fastidiosa* grown in different media. RT-PCR reactions (a) using the *pil*S primers; (b) using the *xps*L primers and (c) using the *xps*E primers. *X. fastidiosa* was grown in (1) Defined medium XDM*; (2) XDM* + sweet orange extract (Ci); (3) XDM* + 'Ponkan' extract (Po); (4) XDM* + coffee extract (Ca) and (5) PW medium. M.1 kb molecular weight (Invitrogen, USA) markers.

described in Mehta and Rosato (2001). *C. sinensis* and *C. arabica* are compatible hosts of *X. fastidiosa* whereas *C. reticulata* has been considered tolerant (Laranjeira et al. 1998).

RNA was extracted from the cells submitted to different treatments using the hot phenol method, which consists, briefly, of a lysis reaction using lysozyme (0.5 mg/ml) and SDS (1%), followed by hot phenol (pH 5) and chloroform extractions. The sample was then precipitated using ethanol and sodium acetate (3 M, pH 5.2) at −70 °C. The RNA was treated with DNAse (10 U) for 2 h at 37 °C. The concentration of total RNA was estimated by using denaturing agarose gel electrophoresis of the 16S and 23S products. The RNA was used in RAPD reactions to verify DNA contamination in the samples. Synthesis of cDNAs was performed using random primers (Gibco-BRL, USA) and 0.5 U of reverse transcriptase (SuperScriptII, Invitrogen, USA), and the final concentration was estimated by RT-PCR amplification using 16S primers. The following specific primers were designed to amplify each gene (see Figure 1) and they were used in the RT-PCR reactions, *pil*S: 2546petD: 5′GGTATTGAGGGT CGCTTGCCT GGTATTCG 3′ and 2546petR: 5′AGAGGAGAGTTAGAGCCTCACTTCGGA AT 3′; *xps*L: 1524petD: 5′GGTATTGAGGGTC GCTTGGGAAGAGGG3′ and 1524petR: 5′AGA GGAGAGTTAGAGCCTTAACGTTGTGCC3′ and *xps*E 1517petD: 5′GGTATTGAGGGTCG CGTGGAAAATAGG3′ and 1517petR: 5′AGA GGAGAGTTAGAGCCTCAGCCTTCCTCCG-TC3′.

The RT-PCR reactions were performed in a final volume of 25 μl comprising 50 ng cDNA, 1X buffer, 120 μM dNTP mix; 10 pmol of primers and 2.5 U of *Taq* DNA polymerase. The reactions were carried out in a MJ Thermo-Cycler (USA) using the cycling conditions of 3 min at 94 °C and 30 cycles of 1 min at 94 °C, 2 min at 60 °C and 3 min at 72 °C.

pilS *analysis*

pilS encodes a histidine kinase sensor protein involved with fimbriae biosynthesis. The amino acid sequence of the protein shows 36% of identity with the *pilS* of *Pseudomonas aeruginosa*. In this bacterium the fimbriae are involved in several processes including adhesion to epithelial cells, which is an important event during colonization (Boyd 2000). Although the signal responsible for *pilS* activation is unknown, previous reports suggest that its transcription could be related to a nutritional state which requires adhesion activity (Wu and Kaiser 1997; O'Toole and Kolter 1998; Boyd 2000). A *pilS* transcript of the expected size (1.8 kB) was detected in *X. fastidiosa* grown in rich and XDM* media supplemented with plant extracts but not in unsuplemented XDM* medium (Figure 2a).

These results indicate that *pil*S might be modulated by nutritional factors, as has been suggested previously (Wu and Kaiser 1997). In richer media, the growth rate of *X. fastidiosa* is higher and the presence of fimbriae could be important in cellular adhesion, which would explain the transcription of *pilS* in amended media. *X. fastidiosa* grows firmly adhered to the flask walls *in vitro* forming biofilms, and *in vivo* it attaches to the xylem vessels (Lemos et al. 2003) possibly preventing sap flow-through in the xylem. In *E. coli* K-12, cell–cell adhesion factors are essential in the biofilm formation (Reisner et al. 2003).

xps *genes*

The *xpsEL* genes belong to a cluster of genes related to a type II secretion system, which is responsible for delivering extracellular enzymes such as cellulases. The transcription analysis of *X. fastidiosa* genes revealed that both genes were expressed when the bacterium was grown in rich medium (PW) and also in XDM* media amended with 'ponkan' (Po) and coffee (Ca) plant extracts, but not in XDM*. A major difference was observed in the treatment with sweet orange extract (Ci), where *xpsL* was not detected (Figure 2b). These results suggest that sweet orange extract may modulate *xpsL* transcription, as this gene was not transcribed in this specific treatment. Interestingly the response of *xpsE*, which belongs to the same operon, seems not to be affected in the same manner (Figure 2c). Although the composition of sweet orange extract is currently unknown, plant extracts are composed of sugars, amino acids and phenolic compounds (Kishore and Chand 1976). Future tests using these components separately would help to elucidate the specific regulation of *xps* genes. Transcription analysis of *xps*EL genes cloned into *E. coli* DH5α was also carried out to verify differential regulation and to assist future studies on protein overexpression. The bacterial cells were cultured in different media, as well as in media amended with plant extracts. Interestingly, although *xpsL* was transcribed in every condition tested, *xpsE* was not (data not shown). It is possible that this gene needs additional factors, which are present in *X. fastidiosa* but unavailable in *E. coli*. Indeed, reports of both XpsL and XpsE in *Xanthomonas campestris* have shown that they interact in two-hybrid assays (Lee et al. 2001).

In conclusion, *pilS* and *xpsEL* genes of *X. fastidiosa* can be modulated by environmental factors and their expression is dependent on the nutritional status of the growth medium. Additionally the differential response of *xps*E and *xps*L in sweet orange extract indicates that the control of the operon is complex. In some cases an operon is controlled by different factors, such as the *sbo-alb* operon of *Bacillus subtilis*, which regulates the subtilisin synthesis by nutritional starvation and anaerobiosis (Nakano et al. 2000). Finally it would be interesting to obtain mutants for *pil*S and *xps* genes to verify how these genes are related with pathogenicity and elicitation of symptoms in sweet orange plants.

Acknowledgments

The authors thank Dr. Edmilson R. Gonçalves for assistance, CAPES for the studentship to P.P.C. and FAPESP for financial support.

References

Beretta M.J.G., Harakava R. and Chagas C.M. 1996. First report of *Xylella fastidiosa* in coffee. Plant Dis. 80: 821.

Bhattacharyya A., Stilwagen S., Reznik G., Feil H., Feil W.S., Anderson I., Bernal A., D'Souza M., Ivanova N., Kapatral V., Larsen N., Los T., Lykidis A., Selkov E., Walunas T.L., Purcell A., Edwards R.A., Hawkins T., Haselkorn R., Overbeek R., Kyrpides N.C. and Predki P.F. 2002. Draft sequencing and comparative genomics of *Xylella fastidiosa* strains reveal novel biological insights. Genome Res. 12: 1556–1563.

Boyd J.M. 2000. Localization of the histidine kinase PilS to the poles of *Pseudomonas aeruginosa* and identification of a localization domain. Mol. Microbiol. 36: 153–162.

Costa de Oliveira R.C., Yanai G.M., Muto N.H., Leite D.B., de Souza A.A., Coletta H.D., Machado M.A. and Nunes L.R. 2002. Competitive hybridization on spotted microarrays as a tool to conduct comparative genomic analyses of *Xylella fastidiosa* strains. FEMS Microbiol. Lett. 216: 15–21.

Davis M.J., French W.J. and Schaad N.W. 1981. Axenic culture of the bacteria associated with phony disease of peach and plum leaf scald. Curr. Microbiol. 6: 309–314.

Dow J.M. and Daniels M.J. 2000. *Xylella* genomics and bacterial pathogenicity to plants. Yeast 17: 263–271.

Hopkins D.L. 1989. *Xylella fastidiosa* – Xylem-limited bacterial pathogen of plants. Annu. Rev. Phytopathol. 27: 271–290.

Hopkins D.L. and Purcell A.H. 2002. *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. Plant Dis. 86: 1056–1066.

Kishore V. and Chand J.N. 1976. Resistance of citrus to citrus canker caused by *Xanthomonas citri* – analysis of phenols and sugars. Ind. Phytopathol. 28: 46–47.

Laranjeira F.F., Pompeu J.Jr., Harakawa R., Figueiredo J.O., Carvalho S.A. and Coletta-Filho H.D. 1998. Cultivares e espécies cítricas hospedeiras de *Xylella fastidiosa* em condições de campo. Fitopatol. Bras. 23: 147–154.

Lee H.M., Tyan S.W., Leu W.M., Chen L.Y., Chen D.C. and Hu N.T. 2001. Involvement of the XpsN protein in formation of the XpsL–XpsM complex in *Xanthomonas campestris* pv. *campestris* type II secretion apparatus. J. Bacteriol. 183: 528–535.

Lemos E.G.M., Alves L.M.C. and Campanharo J.C. 2003. Genomics-based design of defined growth media for the plant pathogen *Xylella fastidiosa*. FEMS Microbiol. Lett. 219: 39–45.

Machado M., Souza A.A., Coletta Filho H.D., Kuramae E.E. and Takita M.A. 2001. Genome and pathogenicity of *Xylella fastidiosa*. Mol. Biol.Today 2: 33–43.

Mehta A. and Rosato Y.B. 2001. Differentially expressed proteins in the interaction of *Xanthomonas axonopodis* pv. *citri* with leaf extract of the host plant. Proteomics 1: 1111–1118.

Mircetich S.M., Lowe S.K., Moller W.J. and Nyland G. 1976. Etiology of almond leaf scorch disease and transmission of causal agent. Phytopathology 66: 17–24.

Nakano M.K.Z., Zheng G. and Zuber P. 2000. Dual control of *sbo-alb* operon expression by the Spo0 and ResDE systems of signal transduction under anaerobic conditions in *Bacillus subtilis*. J. Bacteriol. 182: 3274–3277.

Nunes L.R., Rosato Y.B., Muto N.H., Yanai G.H., da Silva V.S., Leite D.B., Gonçalves E.R., de Souza A.A., Colleta-Filho H.D., Machado M.A., Lopes S.A. and de Oliveira R.C. 2003. Microarray analysis of *Xylella fastidiosa* provide evidence of coordinated transcription control of laterally transferred elements. Genome Res. 13: 570–578.

O'Toole G.A. and Kolter R. 1998. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. 30: 295–304.

Parkinson J.S. 1993. Signal transduction schemes of bacteria. Cell 73: 857–871.

Reisner A.H., Haagensen J.A., Schembri M.A., Zechner E.L. and Molin S. 2003. Development and maturation of *Escherichia coli* K-12 biofilms. Mol. Microbiol. 48: 933–946.

Rossetti V., Garnier M.B., Beretta J.M., Teixeira M.J.G., Quaggio A.R.R. and Negri J.A. 1990. Presénce de bactéries dans lê xyléme d'orangers atteints de chorose variegée, une nouvelle maladie dês agrumes au Brésil. Comp Rend Acad Sci series 3: 345–349.

Russel M. 1998. Macromolecular assembly and secretion across the bacterial cell envelope: Type II protein secretion systems. J. Mol. Biol. 279: 485–499.

Salmond G.P.C. 1994. Secretion of extracellular virulence factors by plant- pathogenic bacteria. Annu. Rev. Phytopathol. 32: 181–200.

Simpson A.J.G., Reinach F.C., Arruda P., Abreu F.A., Acencio M., Alvarenga R., Alves L.M.C., Araya J.E., Baia G.S., Baptista C.S., Barros M.H., Bonaccorsi E.D., Bordin S., Bove J.M., Briones M.R.S., Bueno M.R.P., Camargo A.A., Camargo L.E.A., Carraro D.M., Carrer H., Colauto N.B., Colombo C., Costa F.F., Costa M.C.R., Costa-Neto C.M., Coutinho L.L., Cristofani M., Dias-Neto E., Docena C., El-Dorry H., Facincani A.P., Ferreira A.J.S., Ferreira V.C.A., Ferro J.A., Fraga J.S., Franca S.C., Franco M.C., Frohme M., Furlan L.R., Garnier M., Goldman G.H., Goldman M.H.S., Gomes S.L., Gruber A., Ho P.L., Hoheisel J.D., Junqueira M.L., Kemper E.L., Kitajima J.P., Krieger J.E., Kuramae E.E., Laigret F., Lambais M.R., Leite L.C.C., Lemos E.G.M., Lemos M.V.F., Lopes S.A., Lopes C.R., Machado J.A., Machado M.A., Madeira A., Madeira H.M.F., Marino C.L., Marques M.V., Martins E.A.L., Martins E.M.F., Matsukuma A.Y., Menck C.F.M., Miracca E.C., Miyaki C.Y., Monteiro-Vitorello C.B., Moon D.H., Nagai M.A., Nascimento A., Netto L.E.S., Nhani A., Nobrega F.G., Nunes L.R., Oliveira M.A., de Oliveira M.C., de Oliveira R.C., Palmieri D.A., Paris A., Peixoto B.R., Pereira G.A.G., Pereira H.A., Pesquero J.B., Quaggio R.B., Roberto P.G., Rodrigues V., Rosa A.J.D., de Rosa V.E., de Sa R.G., Santelli R.V., Sawasaki H.E., da Silva A.C.R., da Silva A.M., da Silva F.R., Silva W.A., da Silveira J.F., Silvestri M.L.Z., Siqueira W.J., de Souza A.A., de Souza A.P., Terenzi M.F., Truffi D., Tsai S.M., Tsuhako M.H., Vallada H., Van Sluys M.A., Verjovski-Almeida S., Vettore A.L., Zago M.A., Zatz M., Meidanis J. and Setubal J.C. 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. Nature 406: 151–157.

Wells J.M., Raju B.C., Hung H.Y., Weisburg W.G., Mandelcopaul L. and Brenner D.J. 1987. *Xylella fastidiosa* gen-nov sp-nov – Gram-negative, xylem- limited, fastidious plant bacteria related to *Xanthomonas*-spp. Int. J. Syst. Bacteriol 37: 136–143.

Wu S.S. and Kaiser D. 1997. Regulation of expression of the *pil*A gene in *Myxococcus xanthus*. J. Bacteriol. 179: 7748–7758.