Minireview

From rags to riches: insights from the first genomic sequence of a plant pathogenic bacterium

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Abstract

The recently published genomic sequence of *Xylella fastidiosa* is the first for a free-living plant pathogen and provides clues to mechanisms of pathogenesis and survival in insect vectors. The sequence data should lead to improved control of this pathogen.

Simpson *et al*. [1] recently announced the complete genomic sequence of *Xylella fastidiosa*, a pathogen that causes important diseases in citrus trees, grapevines and other plants [2]. This work, accomplished by a consortium of Brazilian scientists, is the first complete sequence of a plant pathogenic bacterium to be publicly disclosed and, as such, rates as an important development. Ironically, *Xylella* is essentially uncharacterized by classical biochemical and genetic approaches, when compared with several other bacterial plant pathogens. The sequence data, therefore, instantly elevate *X. fastidiosa* from a virtually unknown organism to one for which strong clues are now available to help deduce mechanisms of plant and insect survival and devise control measures.

X. fastidiosa encompasses a number of bacterial strains that infect the xylem elements of higher plant hosts and cause economically important diseases [2]. For instance, two of us (DAC and CKD) are involved with research on a strain causing Pierce's disease of grapevines (leaf scorch, blight and vine die-back), currently threatening wine grapes in California. The bacteria are disseminated and introduced into new plant hosts by insects, namely leafhoppers. As the name implies, the fastidious bacteria are not easily cultured on laboratory media, a result that seems at odds to their growth in the nutrient-poor xylem elements of plants.

Previous 16S rRNA analysis [3] and some of the genes identified by Simpson *et al*. [1] suggest that *Xylella* is most closely related to the plant pathogenic genus *Xanthomonas*, but the *X. fastidiosa* genome is much reduced in size and has a considerably lower GC content. This presumed evolutionary genome reduction may be related to the fact that *Xylella* does not appear to be found in several alternative habitats, in contrast to many other plant pathogenic bacteria, including *Xanthomonas* [4]. But, in addition to reductive evolution in genome size while becoming a xylem specialist, the bacterium has also acquired new genes. The presence of several genes known previously only in animal pathogens (especially those associated with bacteriophage sequences) argues for the acquisition of additional functions that may facilitate its attachment and growth in insect vectors.

The *X. fastidiosa* strain sequenced contained two plasmids and a chromosome of approximately 2.7 Mb. A larger than usual number of open reading frames (53%) could not be assigned putative functions by comparison with known genes. Of the identified genes, expected DNA-metabolizing genes were present as well as those involved in metabolic activity and transport. DNA repair genes, such as photolyase and 'SOS' repair polymerases were not present, and certain gluconeogenesis genes appeared to be missing. These

deficiencies may be a consequence of its plant-host environment, but do not immediately explain the difficulties of growing *X. fastidiosa* in culture.

Adhesion of the bacteria to xylem and insect cell surfaces would seem important for a pathogen such as *Xylella*, and the Brazilian groups identified several genes associated with bacterial fimbriae and adhesion proteins known in other bacterial pathogens. Genes encoding type IV and other pilins [5], hemagglutinin-like genes, and other afimbrial adhesins are potential candidates for bacterial adaptation to insect transmissibility through polar (end-on) attachment to leafhopper mouthparts. Three very large (9-10 kb) homologs of hemagglutinin-like proteins from animal pathogens represent one of the relatively few gene families in the bacterium, and their presence as three copies may permit rapid variation in adhesion properties. Identified genes for xanthan gum production presumably account for bacterial clumping and water flow blockage in infected plant xylem elements. These could have important ramifications for bacterial nutrition and survival.

Several genes were identified that are homologous to known virulence genes from animal and plant pathogens. These include hemolysin- and colicin-like genes, as well as several other putative virulence factors, some of them seen for the first time in a bacterial plant pathogen. The work thus extends findings from the past several years which indicated that plant and animal pathogens share much of their virulence machinery [6]. Some of these *Xylella* genes occurred between prophage sequences, suggesting their recent invasion of the genome. Interestingly, genes were also identified that may function in polyketide synthesis. Because polyketide toxins are frequently virulence factors in bacterial pathogens [7], these genes may indicate the occurrence of similar toxins in *X. fastidiosa*. Several of the identified *X. fastidiosa* genes appear to be involved in heavy metal sequestration and drug efflux, as well as in protection from active oxygen species. These genes have probably been recruited as a consequence of plant defense mechanisms against the bacterium, and the use of heavy metals and other pesticides in commercial agriculture [8].

Enzymes attacking the plant cell wall are frequently secreted by pathogenic bacteria, but Simpson *et al.* [1] identified only a frame-shifted polygalacturonase gene and two cellulase genes that might play such a role in *X. fastidiosa*. The apparent lack of pectin-degrading enzymes may be understandable, given that the bacteria do not need plant invasion mechanisms: they are deposited into the xylem by insects. Genes encoding at least four proteases were identified, and one or more of them may account for the previously observed protease activity in bacterial culture fluids [9]. Although Simpson *et al.* [1] identified putative virulence genes from the sequence, the bacterium appears to lack genes encoding resistance-blocking proteins analagous to *yopJ* in the animal pathogen, *Yersinia pseudotuberculosis* [10]. The bacterium also does not have batteries of genes tailored for nutrient procurement from host polymers (such as in *Erwinia chrysanthemi* [11]) and the attendant hazard of host defense 'surveillance' mechanisms. In their rather cloistered environmental niche of xylem vessels, *Xylella* cells have perhaps largely avoided surveillance by general and specific plant defense systems.

Other than the notable absence of type III secretion systems and target proteins [6], the genomic sequence of *X. fastidiosa* specifies all known pathways for the secretion of extracellular virulence factors in Gram-negative bacteria. Homologs of HlyB and HlyD of *Escherichia coli*, both involved in type I secretion of hemolysin, are present. The bacterium may use this pathway to secrete one or more of its proteases, as in *Erwinia* [12]. *X. fastidiosa* also has homologs of the widely distributed type II (general secretory) pathway, made up of the Sec system and the main terminal branch. Thus, homologs of the Xps (Gsp) proteins (D through L, all components of the secretion machinery) of the plant pathogen *Xanthomonas campestris* are found in the genome of *X. fastidiosa*. Extracellular enzymes, such as the two cellulases mentioned previously, may also be secreted by this pathway. The genome of *X. fastidiosa* also includes homologs of the type IV protein secretion pathway, which is typified by T-DNA transfer into chromosomes of plant cells by the pathogen *Agrobacterium tumefaciens*. The 51 kb plasmid also specifies a full complement of type IV secretory protein homologs, which may be involved in plasmid conjugation. A homolog of *mttC* in *Escherichia coli* involved in type V secretion is also present in the *X. fastidiosa* genome. Consistent with the lack of a type III secretion system, the bacterium does not appear to contain any of the so-called 'avirulence gene' proteins identified from other bacterial plant pathogens that are suspected to function as virulence effectors [6].

Some bacterial pathogens utilize complex regulation networks modulating virulence [13]. Although information is incomplete in *Xylella*, the sequence data identified several putative two-component regulators and transcription factors, as well as regulatory genes known to be involved in the control of virulence and pathogenicity in other bacteria. These include members of the two-component AraC, LysR and LuxR families of regulators, as well as sigma factors that control gene expression under specific physiological conditions. A very important observation is the presence of a GacA homolog: GacA has been shown to control virulence in both plant and animal pathogenic bacteria. *X. fastidiosa* also has homologs of RpfB and RpfF, which are involved in the synthesis of diffusible signal factor(s) regulating pathogenicity in *Xanthomonas campestris*. This observation is potentially significant because it could explain how the bacterial cells in the plant xylem or insect mouthparts communicate with each other, to achieve the concerted production of virulence

and/or adhesion factors. Also significant is the presence of a homolog of *rsmA* (*CsrA*) which, together with its cognate regulatory RNA (*rsmB* or *csrB*), post-translationally controls numerous traits, including pathogenicity, in diverse bacteria. The presence of *rsmA* (and possibly *rsmB*) homologs suggests that *X. fastidiosa* may also regulate gene expression post-transcriptionally.

So, where to go from here? Progress will require that basic molecular technology be developed for the bacterium, a task which the Sao Paulo groups are doubtless feverishly undertaking. Naturally, it would be hoped that cloning plasmids useful for driving gene expression in *Xylella* could be constructed, and gene knockout techniques developed in order to test the functions of the various predicted open reading frames. Microarray gene expression strategies for comparing gene expression in culture to that when cells are grown in plant xylem or insect mouthparts will also be informative. As a step towards these goals Simpson *et al*. have provided a website with tools for searching and mapping the *Xylella fastidiosa* genome sequence and identifying putative gene functions [14]. The future promises greater advances for *Xylella* research, a field that is certainly far richer than before publication of the complete genome sequence.

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