Towards identification of pathogenicity genes specific to *Xanthomonas albilineans* strains closely associated with sugarcane leaf scald disease outbreaks.

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Xanthomonas albilineans is a xylem-invading pathogen that causes leaf scald, one of the major diseases of sugarcane. Interestingly, this pathogen lacks both the xanthan gum genes cluster and a type III secretion system (T3SS) of the Hrp1 and Hrp2 injectisome families (1). *X. albilineans* produces a unique and specific toxin, albicidin, which is a powerful DNA gyrase inhibitor. Consequently, albicidin blocks chloroplasts differentiation, resulting in leaf scald symptoms. High genetic and pathogenic variability exists among strains of *X. albilineans*, and 10 genetic groups were identified by pulsed field gel electrophoresis (PFGE). All strains involved in sugarcane leaf scald disease outbreaks since the late 1980s belong to the same genetic group called PFGE-B, whereas the strains isolated previously belong to other groups, especially to group PFGE-A. These two groups were also revealed by multilocus sequence analysis (MLSA) using seven housekeeping genes (*groEL, dnaK, gyrB, atpD, efp, recA and glnA*). The complete genome sequence of strain GPE PC73 belonging to PFGE-B is now available (1). To better understand the genetic differences between the PFGE-A and PFGE-B strains, Suppression Subtractive Hybridization (SSH) analysis was performed to compare the genomes of XaFL07-1 (PFGE-B) and Xa23R1 (PFGE-A), both isolated in Florida. SSH is a method used to identify DNA fragments that are uniquely found in one strain when compared with another, closely related bacterial strain (2,3). We enriched a library of unique DNA sequences from strain XaFL07-1 (tester strain), using Xa23R1 DNA as the driver strain. A total of 188 XaFL07-1-specific clones were generated and sequenced. Sequences were all compared against the GPE PC73 genome and against the GenBank non redundant database (NCBI). Initial screening focused on 12 genes with potential pathogenicity function and for which SSH data were confirmed by PCR and Southern blot hybridization. These included a DNA methyltransferase, a chemotaxis protein, a permease, a CRISPR protein, and an Rhs protein. However, after further distribution studies of these genes among strains of *X. albilineans* representing the genetic diversity observed in this pathogen, only one gene encoding a DNA (cytosine-5)-methyltransferase was found to be specific to PFGE-B strains. In GPE PC73, this gene is localized in a 53 kb chromosome region of phage origin that includes also two other DNA methyltransferases. SSH often results in identification of phage DNA (3); DNA methyltransferases are known to play a role in genetic regulation by modifying the binding of DNA polymerases on promoters. Additional PCR and Southern blot screening of all SSH clones, including genes with unknown functions, could also lead to the identification of other pathogenicity genes specific to PFGE-B strains of *X. albilineans*. Knockout mutagenesis of the DNA methyltransferases and of other SSH candidate genes will be performed to investigate the role of these genes in pathogenicity of *Xanthomonas albilineans* strains closely associated with sugarcane leaf scald disease outbreaks.

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