IDENTIFICATION OF GENES INVOLVED IN TWITCHING MOTILITY IN *Acidovorax citrulli* BY GENERATION AND CHARACTERIZATION OF A LIBRARY OF TRANSPOSON MUTANTS

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ABSTRACT

Bacterial fruit blotch (BFB) of cucurbits is a devastating disease caused by *Acidovorax citrulli*, a Gram-negative bacterium transmitted through infested seed (Schaad et al., 2008). Serious economic losses caused by *A. citrulli* in cucurbits have been reported in many parts of the world (Burdman & Walcott, 2012). Because of the lack of efficient methods to control BFB, this disease represents a serious threat to the cucurbit industry, and primarily of watermelons and melons. Despite the economic importance of BFB, the knowledge of the mechanism of the basic biology of plant-pathogen interaction of *A. citrulli* is still very much challenging and limited. This has been a limiting factor towards the development of efficient management procedures to efficiently combat BFB. Despite the multiple functionality of type IV pili (T4P) and their well-established role in the pathogenicity of animal pathogenic bacteria relatively little attention has been given to the role of T4P in plant-pathogenic bacteria (Burdman et al., 2011). The knowledge of the genes involved with the T4P that contributes strongly to the bacterium virulence and twitching motility will go a long way in helping to design an appropriate method for the disease management. To identify the genes involved in twitching motility we generated two libraries of transposon mutants of *A. citrulli* M6 using the EZ-Tn5 technology (Epicenter). Twitching motility mutants of both libraries were screened three times for altered twitching motility phenotype by plating onto Nutrient agar (NA) with 50 ppm kanamycin (Na/Km50) and were assessed with the naked eye for alteration in twitching motility. To identify the mutated region of each selected mutant, a rescue clone procedure was applied. The genomic DNA of the mutant was purified and digested with an enzyme (Sacl or Pael) that does not cut inside the mutagenesis cassette, keeping the Km gene and the R6Kγ ori fragment intact. Digestion products were then autoligated and electroporated into *E. coli* BW25141 cells and plated onto Luria Bertani (LB) broth and Bacto agar with 50 ppm kanamycin LB/Km50. Km resistant (KmR) transformants were purified using a miniprep kit, and the products were sent to Hylabs for
sequencing of the flanking regions surrounding the insertion site of the mutagenesis cassette using pMOD<MCS> primers. The results of sequenced DNA of each mutant are being compared with the genome sequence of the *Acidovorax citrulli* group II strain AAC00-1 using the NCBI Blast tool. Results from the assessment of mutants with the naked eye lead to the detection of thirty-two mutants with altered twitching motility from the libraries out of which ten have been sequenced and the genes involved in the twitching motility have been identified. The genes identified include *pilW*, putative transmembrane protein (identical to the *FimV* in *Acidovorax avenae* subsp. *avenae*), general secretion pathway protein, *CheA*, Sigma 54 subunits, type IV prepilin peptidase 1, *FlgC*, a Duf21 protein, 50s ribosomal protein L10 and ATPase subunit HsIV. Results from the seed transmission assays on melon shows significant (p=0.05) differences between the mutants and wild type M6, with the mutants showing reduced levels of virulence as compared to the wild type. This result suggests the importance of the listed genes, demonstrate their involvement in twitching motility and stress the significant contribution of T4P and twitching motility for *Acidovorax citrulli* virulence.

More research work is still ongoing in identifying the genes involved in twitching motility in *A. citrulli* and their respective role in the virulence of the bacterium.

References


Burdman, S., R. Walcott. 2012. *Acidovorax citrulli* : Generating basic and applied knowledge to tackle a global threat to the cucurbit industry. Molecular plant pathology 13(8), 805-81


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