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Characterization of the quorum -sensing system of a biocontrol strain *Burkholderia ambifaria* from maize rhizosphere

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Abstract

The aim of the present research was to characterize the quorum-sensing system of a maize rhizosphere isolate of *Burkholderia ambifaria*, strain BcF, isolated at the USDA Plant Protection Lab in Beltsville, MD, and found to exhibit antifungal activity against a variety of fungal pathogens. I found that strain BcF produced high levels of *N*-acyl homoserine lactones (AHLs) compared to closely related species of the *Burkholderia cepacia* complex. The predominant AHL was octanoyl homoserine lactone. A major goal of the project was to isolate mutants of strain BcF blocked in AHL formation and examine whether functions related to biocontrol were subject to quorum-sensing regulation. ^ I used PCR technology to isolate a 2,191-nt DNA fragment containing two divergently oriented genes, *bafI* and *bafR*, encoding, respectively, AHL synthase and AHL-binding transcriptional activator. Putative promoters were identified for both genes. The 724-bp intergenic region between *bafI* and *bafR* contained a 417-nt ORF of unknown function, which may be cotranscribed with *bafR*. ^ AHL-deficient strains, in which the *bafI* and *bafR* genes had been inactivated by allelic exchange or by transposon mutagenesis, exhibited decreased exoprotease and antifungal activity, and overproduced siderophores. I also isolated spontaneous AHL-deficient mutants with the same phenotypes in which the *bafIR* locus had been deleted. The AHL-deficient mutants had reduced ability to inhibit the growth of fungal pathogens such as *Pythium ultimum*, *Rhizoctonia solani*, and *Fusarium oxysporum*, and also exhibited reduced ability to suppress damping-off of cucumber by *P. ultimum* *in vivo*. We have constructed *gfp*-labelled derivative of strain BcF that we plan to use to monitor root colonization *in vivo*. We are attempting to isolate AHL-deficient mutants of this strain that can be used to examine the influence of AHLs on root colonization. ^ Pulsed-field gel electrophoretic analysis of randomly linearized replicons from strain BeF indicated that it contained three chromosomes of 3.5, 2.8 and 1.3 Mb and had an overall genome size of 7.6 Mb. Macrorestriction fragment mapping of the site of transposon insertion in a *bafR* mutant as well as Southern hybridization experiments indicated that the *bafIR* locus was located on the 2.8-Mb chromosome. ^

Subject Area

Agronomy|Microbiology

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