APPLICATIONS OF GENOMICS TO BIOLOGICAL CONTROL OF SOILBORNE DISEASES

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The power of genomics is being applied to biological control by sequencing the complete genome of the biological control organism *Pseudomonas fluorescens* Pf-5. Strain Pf-5 was selected as a model biocontrol strain for a genomic sequencing project because of its success as a biocontrol agent, its use as a standard biocontrol strain by many researchers, and the body of knowledge that exists on the mechanisms by which Pf-5 suppresses soilborne plant disease.

Pf-5 was first described for its capacity to suppress soilborne diseases of cotton caused by *Rhizoctonia solani* (Howell and Stipanovic 1979) and *Pythium ultimum* (Howell and Stipanovic 1980). Since it was first described, Pf-5 has been shown to suppress these pathogens on plant hosts including cucumber, pea, and maize. Pf-5 also suppresses a number of other soilborne or residue-borne pathogens such as *Pyrenophora tritici-repentis*, *Sclerotinia homoeocarpa* and *Dreschslera poae*, *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Erwinia carotovora*.

Pf-5 produces a suite of antibiotics including pyrrolnitrin (Howell and Stipanovic 1979), pyoluteorin (Howell and Stipanovic 1980; Nowak-Thompson et al. 1999), and 2,4-diacetylphloroglucinol (Nowak-Thompson et al. 1994). Each of these compounds has a different spectrum of activity against plant pathogens, and their roles in biological control have been established in various biological control organisms. Pf-5 is remarkably similar to *P. fluorescens* strain CHA0, a rhizosphere bacterium isolated in Switzerland, in its biological control activities and its antibiotic production. Strains Pf-5 and CHAO have been developed as model organisms for the study of biological control, rhizosphere biology, and antibiotic regulation in Gram-negative bacteria, and the collective knowledge accumulated on these strains is unsurpassed among environmental isolates of *Pseudomonas* spp.

Genomic sequencing of Pf-5 began in March of 2002 at The Institute of Genomics Research, under the direction of Dr. Ian Paulsen, and was completed in September of 2003. Currently, the genome is being annotated to assign functions to as many genes in the genome of Pf-5 as possible. Completion of genome annotation is expected in spring of 2004.

The genome of Pf-5 is estimated at 7.3 Megabases, which is 0.6 to 1.3 Megabases larger than genomes of other *Pseudomonas* spp. that have been sequenced to date. As expected from the spectrum of antibiotics produced by Pf-5, many biosynthetic gene clusters for secondary metabolite production are present in the genome. The prevalence of large gene clusters for secondary metabolite production must contribute to the large size of the Pf-5 genome. Genes known to contribute to biological control of Pf-5 are distributed throughout the genome; they are not clustered in groups analogous to the pathogenicity islands found in pathogenic *Pseudomonas* spp. There is no evidence for the presence of genes involved in pathogenicity (ie. phytotoxins, Type III secretion systems, effectors, etc.) in the genome of Pf-5; such genes are found in the genomes of phytopathogenic *P. syringae.* Therefore, there are significant differences in the genomes of phytopathogenic and biocontrol strains of *Pseudomonas* spp. Twenty one genes encoding outer-membrane receptors for siderophores or other iron-uptake systems are found in the genome of Pf-5, suggesting the importance of iron-acquisition in the ecology of this bacterium.

Post-genomics technology offers great promise for enhancing knowledge of biological control. To this end, we have developed a low-density microarray in which factors influencing the expression of genes required for biological control are being evaluated. Knowledge of factors influencing the expression of biological control genes is needed to understand the currently-unexplained variation in biological control that can occur from field to field. Variable efficacy poses a serious obstacle to the development of biocontrol products by industry and the use of these products by growers. More reliable biocontrol products and processes can be developed if sources of variation are identified and strategies for decreasing variability are implemented.

For more information, visit the Pf-5 genome website:
http://www.ars-grin.gov/hcrl/Pf5genome/index.htm

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