Genetic Basis of Biological Control in a Bacterium Antagonistic to Turfgrass Pathogens

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Goals:

- Identify and clone DNA sequences that encode pathogen-suppressive properties in *Enterobacter cloacae*.
- Determine the nucleotide sequence of *E. cloacae* DNA encoding pathogen-suppressive properties and tentatively establish a function for the gene product.
- Evaluate, in field studies, the expression of the biocontrol-related gene, *psp1*, under typical turfgrass management conditions.
- Identify nucleotide sequences of *E. cloacae* DNA encoding for pathogen suppression.

The purpose of this project on *Enterobacter cloacae* genetics is to determine the array of bacterial traits responsible for biological control activity in bacteria. Our focus has been on *Pythium*-incited disease of creeping bentgrass, but we believe our studies will have broad applicability to other bacterium-pathogen interactions.

Our studies in 1994 focussed primarily on the identification of nucleotide sequences of *E. cloacae* DNA encoding for pathogen suppression. Prior to the initiation of the work, we had isolated mutant V58 which was unable to suppress *Pythium ultimum* seed rot of cucumber. We were able to verify that, whereas the wild-type strain, EcCT-501, was an effective biological control agent of *Pythium graminicola* on creeping bentgrass, mutant V58 was an ineffective biological control agent against this pathogen. Our work subsequently was focussed on the molecular and physiological characterization of mutant V58.

We have inserted the wild-type DNA into the mutant strain V58 and effectively restored all of the biological phenotype of the wild-type strain. Using conventional molecular techniques, we have been able to isolate and sequence portions of the disrupted gene in mutant V58. From sequence analyses, we have discovered that the gene shares high homologies, both at the nucleotide and amino acid levels, with malate dehydrogenase from both *Escherichia coli* and *Salmonella typhymurium*. We performed a series of enzyme assays to verify that the gene we had cloned was actually a malate dehydrogenase (Mdh) gene. Clearly, the wild-type EcCT-501 possessed high levels of Mdh activity. This activity was totally absent in mutant V58.

Furthermore, upon complementation with the putative Mdh gene, Mdh activity was restored. Therefore, we feel confident that we have discovered a malate dehydrogenase gene with a major influence on the biological control of *P. graminicola* on creeping bentgrass and *P. ultimum* on cucumber.