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

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EXECUTIVE SUMMARY

Enterobacter cloacae is an effective biological protectant against infection from many different soilborne plant pathogens. It is particularly effective in suppressing turfgrass diseases incited by *Pythium* species but is also effective against a number of other turfgrass pathogen including *Magneporthe poae* and *Sclerotinia homoeocarpa.* The precise mechanisms of pathogen and disease suppression by *E. cloacae* are as yet unknown, although a number of traits have been empirically-related to the suppression of seed and seedling rots caused by *Pythium ultimum.* To date, however, no conclusive results point to one major mechanism by which *E. cloacae* suppresses diseases caused by *Pythium* species.

An understanding of biological control mechanisms begins with an understanding of the host-pathogen interaction targeted for control. One of the more important aspects of *Pythium* diseases of plants is that *Pythium* species respond extremely rapidly to germinating seeds and growing roots. Since *Pythium* spp. are highly dependent on exudate molecules to initiate these rapid responses to and infection of plants, microbial interference with the production and activity of such stimulatory molecules could be an effective mechanism of biological control of *Pythium* diseases.

Over the past few years we have been exploring the possibility of such a mechanism operating with *E. cloacae* and its suppression of *Pythium* diseases. Our previous research with other crop plants has shown that *E. cloacae* and other seed-applied rhizobacteria can utilize seed exudate from a variety of plant species as a sole carbon and energy source and, at the same time, rapidly reduce the stimulatory activity of exudate to *P. ultimum* sporangia. Depending on the cell density, this inactivation of exudate can occur as rapidly as 2-4 hr.

Other previous work in our laboratory indicated that unsaturated long chain fatty acids (LCFA) found in plant exudates were the primary molecules responsible for the elicitation of *Pythium* responses to plants. From analysis of these exudate fatty acids, we have found linoleic acid to be the most abundant unsaturated fatty acid found in exudates from a number of plant species, including creeping bentgrass and perennial ryegrass.

In an initial survey of bacteria recovered from seeds of various plant species, we found that few of these bacterial strains could reduce the stimulatory activity of perennial ryegrass exudate within 24 hr to levels capable of inducing less than 30% *Pythium* sporangium germination. However, of those strains with activity, the majority were strains of *Enterobacter cloacae.* There were also good correlations between the ability of *E. cloacae* strains to inactivate linoleic acid and their ability to protect creeping bentgrass from Pythium damping-off.

We have taken a multifaceted approach involving physiological, biochemical, and molecular studies to more closely investigate the relationship between fatty acid metabolism and biological control processes. Wild-type strains of *E. cloacae* can utilize a variety of both unsaturated and saturated LCFA as sole carbon and energy sources but grow very poorly, if at all, on medium-chain length (C_7-C_{11}) fatty acids. In addition, *E. cloacae* is capable of eliminating the stimulatory activity of unsaturated LCFA to *P. ultimum* sporangia in as little as 12 hr.

In our studies, we have taken advantage a molecular genetic approach of generating mutants deficient in fatty acid metabolism so that relationships between this function and biological control activity can be observed. In initial studies, we were able to generate a number of mutants of *E. cloacae* strain EcCT-501 that were no longer suppressive to *P. ultimum* in creeping bentgrass assays. It turned out, however, that these were largely mutations in genes encoding TCA (tricarboxylic acid) and DCA (dicarboxylic) cycle enzymes.

More recently we have been able to generate mutants specifically dysfunctional in the ß-oxidation of fatty acids. The first mutant obtained (strain Ec31) fails to grow on media containing linoleic acid as a sole carbon source, but grows well on a minimal media containing succinate. This selection protocol was chosen to avoid selecting mutants with disrupted TCA and DCA cycle enzymes. This mutant is unable to reduce the stimulatory activity of linoleic acid, very slowly reduces the stimulatory activity of seed exudate, and fails to protect bentgrass seedlings from infection by *Pythium* species. Subsequent