

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

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Goals:

- *Identify and clone genes involved in fatty acid metabolism in E. cloacae strain EcCT-501.*
- *Sequence fatty acid metabolic genes.*
- *Establish relationships between fatty acid metabolism and biological control of Pythium-incited diseases on creeping.*

Cooperator:

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The main goal of our project on *Enterobacter cloacae* genetics has been to identify the genetic determinants for biocontrol traits in *Enterobacter cloacae* so that their role in the suppression of *Pythium*-incited diseases of turfgrasses can be specifically elucidated. However, in the last year, we have had to modify our objectives slightly because of recent findings on the nature of pathogen suppression in this system.

In 1996, we obtained several additional mutants. The most notable of these are strains 3-1 and 4-1 that fail to grow on media containing linoleic acid as a sole carbon source, but grow well on a minimal media containing succinate. This selection protocol was chosen to avoid selecting mutants with disrupted Krebs cycle enzymes. As with mutants V58 and 21-1, mutants 3-1 and 4-1 are unable to reduce the stimulatory activity of linoleic acid, exudate from seed, and to protect bentgrass seedlings from infection by *P. ultimum*.

Subsequent complementation and sequence analysis has revealed that the mutation in strain 3-1 is in the *fadAB* operon, which encodes five structural genes central to the β -oxidation of fatty acids. While this mutant is severely debilitated in its ability to catabolize linoleic acid, it is not clear whether this mutation represents deficiencies in linoleic acid transport or in linoleic acid utilization. Therefore, we feel that a search for *fadL* and *fadD* mutants are central to our work.

We have spent considerable effort over the past year trying to sequence the entire

fadAB operon. We currently have the entire region sequenced upstream of the transposon insertion whereas the downstream portion is nearly 80% sequenced. We are currently in the process of trying to generate *fadL* and *fadD* mutants to allow us to ask questions about the role of fatty acid transport and utilization in biological control processes.

We currently feel we have strong laboratory evidence for the role of fatty acid metabolism in biological control processes with *Pythium* species on turfgrasses. Our work will focus over the next few years in trying to 1) determine whether these processes do indeed function in turfgrass soils; 2) further identify *fadL* and *fadD*

mutants that will help us to distinguish between fatty acid uptake and utilization; 3) continue the sequencing of fatty acid genes; and 4) examine turfgrass species and varieties for fatty acid levels in seeds.

The knowledge that the inactivation of fatty acid germination stimulants could be an important mechanism by which bacterial biocontrol agents interfere with pathogens may have an influence on the screening methods for effective biocontrol organisms, since organisms best capable of inactivating stimulants could be selected. Development of turfgrasses that produce seed with low fatty acid content could help seedlings become less susceptible to certain soil-borne pathogens.

Table 6. Differential protection of creeping bentgrass from infection by different *Pythium* species by wild-type, mutant, and complemented strains of *Enterobacter cloacae*.

<i>E. cloacae</i> strain	Disease Rating 1-5 Scale		
	<i>P. ultimum</i>	<i>P. graminicola</i>	<i>P. aphanidermatum</i>
EcCT-501 (WT)	1.8*	1.3*	2.0*
3-1	4.0	5.0	3.3*
4-1	5.0	5.0	5.0
Non-treated	5.0	5.0	5.0
Uninoculated	1.0	1.0*	1.0*

Means followed by (*) are significantly different from non-treated plants according to T-tests.

Rating scale: 1 = healthy turf and 5 = 100% unemerged or necrotic.

Ratings were determined 7 days after inoculation.