

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

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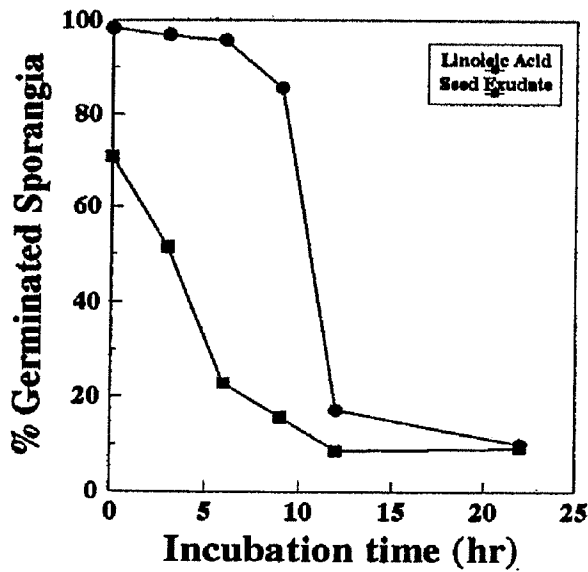
Goals:

- 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 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. Even though our focus has been on *Pythium*-incited diseases of creeping bentgrass, we believe our studies will have broad applicability to other bacterium-pathogen interactions.

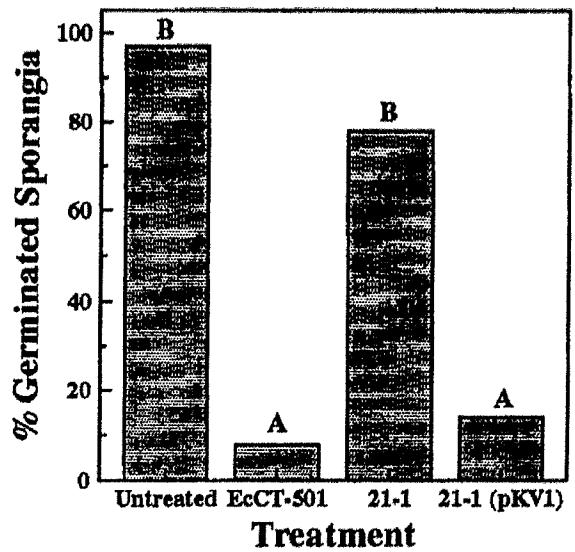
Our studies in 1995 focussed primarily on the first two objectives. During work in 1994, we spent considerable time studying mutant V58, which was a biocontrol negative mutant deficient in malate dehydrogenase activity. We further isolated other mutants lacking significant levels of biological control activity. One such mutant, 21-1, was the focus of our studies in 1995. These studies were concerned with establishing the role of fatty acid metabolism in biological control.

The parent strain of *Enterobacter cloacae*, strain EcCT-501, suppresses several different *Pythium* species, including *P. ultimum*, *P. aphanidermatum*, and *P. graminicola*, on creeping bentgrass. Furthermore, this strain inactivates the stimulatory activity of creeping bentgrass seed exudate, as well as the exudate of many other crop plants, thus preventing responses of these *Pythium* species to plants. With *P. ultimum* in particular, sporangium germination is greatly reduced in the presence of strain EcCT-501. As a result, many of our studies focussed on interactions with *P. ultimum* on creeping bentgrass.



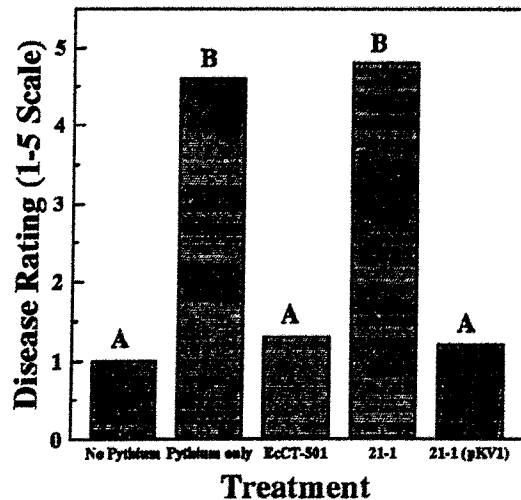
Inactivation of creeping bentgrass seed exudate and linoleic acid solutions by *Enterobacter cloacae* strain EcCT-501.

From among all exudate components, long chain fatty acids (LCFA) are important stimulants of sporangium germination. Our work in 1995 centered on initial attempts to examine the role of LCFA catabolism in the expression of biological control properties in *E. cloacae*. Strain EcCT501 reduced the stimulatory activity of the LCFA, linoleic acid, the most abundant LCFA found in creeping bentgrass and other plant seed exudates. A series of *TnphoA* mutants (Kan^1) were screened for growth on linoleic acid as a sole carbon and energy source. One out of 5000 Kan^1 colonies was deficient in the ability to inactivate the stimulatory activity of creeping bentgrass seed exudate and linoleic acid to *P. ultimum* sporangium germination. Furthermore, this mutant, 21-1, no longer protected creeping bentgrass from *Pythium* seed and seedling disease. A cosmid, PKVI, mobilized into mutant 21-1, complemented the linoleic acid catabolic deficiencies and restored the ability to



Inactivation of the stimulatory activity of linoleic acid by *E. cloacae* strains EcCT-501, 21-1 and 21-1 (pKV1).

inactivate creeping bentgrass seed exudate stimulatory activity. Furthermore, this clone fully restored biological control properties to wild-type levels. Current evidence suggests a role of fatty acid metabolism in biological control properties in *Enterobacter cloacae*.



Disease ratings on creeping bentgrass 6 days after inoculation with *Pythium ultimum*. Disease severity rated on a scale of 1 to 5 for which 1 = healthy seedlings and 5 = 100% necrotic or unemerged.