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BARD

FINAL REPORT

PROJECT NO. IS-2520-95

**The Interaction Between Nonpathogenic Mutants
of *Colletotrichum* and *Fusarium* and the Plant
Host Defense System**

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- **Cooperation**, briefly explain whether synergistic, complementary or supportive.

The cooperation is extremely productive and synergistic. The project has enabled numerous publications and a continuation BARD proposal has been submitted in September 2000.

Abstract

TITLE: The interaction between nonpathogenic mutants of *Colletotrichum* and *Fusarium*, and the plant host defense system.

The intent of this proposal was to study the interaction between nonpathogenic mutants of *Colletotrichum magna* and *Fusarium oxysporum*, and the cucurbit host defense system. We had shown previously that a nonpathogenic endophytic mutant, path-1 of *C. magna*, caused no visible disease symptoms but protected watermelon seedlings from disease caused by the wildtype isolate and *F. o. niveum*. Objectives were: 1) Determine the microscopic, biochemical and molecular genetic interactions between "protected" (path-1 colonized) cucurbit hosts and wildtype isolates of *C. magna*; 2) Isolate non-pathogenic mutants of *F. o. melonis* and test feasibility for protecting plants against fungal diseases. We found that path-1 caused no visible disease symptoms in cucurbit seedlings but conferred disease resistance against pathogenic isolates of *C. magna*, *C. orbiculare*, and *F. oxysporum*. Disease resistance conferred by path-1 correlated to a decrease in the time of activation of host defense systems after exposure of path-1 colonized plants to virulent pathogens. This was determined by monitoring the biochemical activity of PAL and peroxidase, and the deposition of lignin. It appears that path-1-conferred disease resistance is a multigenic phenomenon which should be more difficult for pathogens to overcome than single gene conferred resistance. Based on the benefits conferred by path-1, we have defined this mutant as expressing a mutualistic lifestyle. REMI (restriction enzyme-mediated integration) nonpathogenic mutants were also isolated using pHA1.3 plasmid linearized with *Hind* III and transformed into wildtype *C. magna*. The integrated vector and flanking genomic DNA sequences in REMI mutant R1 was re-isolated and cloned resulting in a product of approximately 11 kb designated pGMR1. Transformations of wildtype *C. magna* with pGMR1 resulted in the same non-pathogenic phenotype.

A nonpathogenic mutant of *F. o. melonis* (pathogenic to melon) was isolated that colonized melon plants but elicited no disease symptoms in seedlings and conferred 25 - 50% disease protection against the virulent wildtype isolate. Subsequently, nonpathogenic mutant isolates of *F. o. niveum* (pathogenic to watermelon) were also isolated. Their protection capacity against the respective wildtype parent is currently under investigation. This research has provided information toward a better understanding of host-parasite interactions; specifically, endophytes, pathogens and their hosts. It will also allow us to assess the potential for utilizing nonpathogenic mutants as biological control agents against fungal pathogens and isolating molecular genetic factors of pathogenicity in *Fusarium*.

Achievements

Significance of main scientific achievements or innovations

The objectives were accomplished as originally stated.

Determine the microscopic and biochemical interactions between “protected” (path-1 colonized) cucurbit hosts and wildtype isolates of *C. magna*

The path-1 mutant did not cause disease symptoms on any cucurbit species but retained the ability to infect and colonize host tissues. In addition, plants colonized with path-1 were protected 100% and 85% against disease caused by virulent isolates of *C. magna* or *Fusarium oxysporum*, respectively, and 90 - 100% against disease caused by *C. orbiculare* (Freeman and Rodriguez, 1993; Redman et al., 1999a). Collectively, these data indicated that the path-1 mutation converted a virulent pathogen to a nonpathogenic endophytic mutualist.

The occurrence of path-1 induced plant protection is dependent on the ability of path-1 to infect and colonize the plants. A host range study revealed that path-1 retained wild type host range but caused no disease on any of the plants tested (Freeman and Rodriguez, 1993; Redman et al., 1999a). From these data, we conclude that in *C. magna*, the genetic basis of pathogenicity and host colonization involve at least some unique genetic elements. Since the path-1 colonized plants are resistant to virulent wild type fungi (*C. magna*, *C. orbiculare*, and *F. oxysporum*) it was unlikely that path-1 suppressed the host defense systems. Biochemical studies indicated that path-1 avoided activation of host defenses and that path-1 colonized plants, challenged with virulent isolates, activated host defenses more quickly than control plants (Redman et al., 1998). These results were determined by monitoring the activity of peroxidase and the deposition of lignin in path-1 colonized and control plants.

Isolation of REMI mutants using a molecular genetic approach

A molecular genetic approach was also taken to isolate nonpathogenic mutants by restriction enzyme-mediated integration (REMI) with the pHA1.3 plasmid linearized with *Hind* III and transformed into *C. magna* isolate L2.5 in the presence

of *Hind* III as described previously (Rodriguez and Redman, 1994). This strategy allowed us to generate 14,400 hygromycin resistant REMI Tx's and 176 non-pathogenic REMI mutants were isolated (Redman and Rodriguez, 2000). Pathogenicity and hybridization data indicated that several different fungal genomic sequences were responsible for the non-pathogenic phenotypes. Two non-pathogenic REMI mutants (R1 and R21) with single site integrations were genetically analyzed suggesting modification of one or more pathogenicity genes. The integrated vector and flanking genomic DNA sequences in REMI mutant R1 was re-isolated and cloned resulting in a product of approximately 11 kb designated pGMR1. A total of 140 Tx's were screened for the loss of pathogenicity and 47% were found to be non-pathogenic and express the same phenotype as the R1 mutant. These results indicate that gene disruption events can occur at a high frequency in *C. magna*.

Isolation of non-pathogenic mutants of *F. o. melonis* and testing feasibility for protecting plants against fungal diseases.

UV-mutagenesis was conducted on wildtype isolate race 1/2 of *F. o. melonis* using the previously described method (Freeman and Rodriguez, 1992). Approximately 800 survivors of UV-mutagenesis were screened for pathogenicity on a set of differential cultivars. Of these, isolate 4/4 was completely nonpathogenic and did not cause any disease symptoms using the dip-inoculation screening method (Freeman and Rodriguez, 1993b). Isolate 4/4 colonized a variety of melon cultivars as well as watermelon seedlings at varying degrees. In cross-protection experiments with melon cultivars no significant differences were observed between 4/4 colonized challenged plants compared with challenged plants alone. However, isolate 4/4 (originally from melon) was able to reduce wildtype mortality of watermelon seedlings (cv. Odem and Malali) caused by *F. o. niveum* race 2. Colonization percent corresponded to percent of protection.

Potential for application

The nonpathogenic mutants isolated and "created" are potentially novel biocontrol agents. To date no biocontrol agents have been used that were derived from wildtype pathogenic isolates. For example, in *Fusarium*, only saprophytes have

been utilized for biocontrol. These mutants therefore may compete better than their “parents” when applied in the correct manner.

Other benefits

Besides the potential use as biocontrol agents, the non-pathogenic mutants will enable a better understanding of the genes involved in pathogenicity. Future work will focus on cloning the flanking regions of pGMR1 and isolating the respective gene(s) involved in pathogenicity. Complementation of the isolates path-1 of *C. magna* and 4/4 of *F. o. niveum* with wildtype genomic libraries, will also enable us to characterize the genes involved in pathogenicity may allow for development of better control measures.

Details of cooperation

The objectives of the proposal were successfully accomplished due to the numerous meetings that took place during the duration of the proposal. The Israeli and US lab personnel were able to meet twice during the course of 1998 at a BARD supported *Colletotrichum* workshop in Israel in August/September and in Seattle in November. Experiments were planned resulting in a joint publication in Plant Physiology. A further meeting took place at the Fungal genetics meeting in the spring of 1999 to discuss the submission of a continuation proposal to BARD based on our accomplishments to date.

Technical Format

Background, scientific and agricultural relevance of the project

Few, if any, plants in natural ecosystems exist independent of symbiotic associations with mycorrhizal and/or endophytic fungi (Petrini, 1986). In the last several decades it has become apparent that symbiotic fungi are important for the structure, function, and health of plant communities (Bacon and Hill, 1996; Clay and Holah, 1999; Read 1999; Read and Camp, 1986; Rodriguez and Redman, 1997). Without these symbiotic associations, it is possible that plant communities may not survive many environmental stresses.

Fungal symbionts express a variety of symbiotic lifestyles including mutualism, commensalism, and parasitism. Mutualistic symbioses have been

shown to confer several benefits to plants such as drought tolerance (Bacon, 1993; Read and Camp, 1986), metal tolerance (Read, 1999), enhanced growth (Belesky et al., 1987; Marks and Clay, 1990; Varma et al., 1999), disease resistance (Carroll, 1986; Freeman and Rodriguez, 1993; Redman et al., 1999a), herbivore resistance (Latch, 1993), and nutrient acquisition (Read, 1999). Commensalistic symbioses have no apparent beneficial or detrimental outcomes of the association. The impacts of parasitic fungi range in severity from decreased growth rates and/or fecundity, to inducing disease symptoms and lethality (described here as a pathogenic lifestyle).

Extensive efforts have been invested in understanding the genetic and biochemical processes that result in parasitism, however, comparatively little effort has gone into understanding mutualistic or commensalistic symbioses. By understanding the genetic and biochemical processes required for colonization and lifestyle expression it may be possible to establish symbioses for improving plant tolerance to environmental stresses and therefore, design more effective strategies for agricultural productivity. In addition, the ability to confer disease resistance to plants via mutualistic symbioses may provide environmentally safe biological alternatives to chemical pesticides that have negative environmental impacts.

The genus *Colletotrichum* contains pathogenic filamentous fungal species that vary extensively in host range with some infecting only a single plant species while others colonize more than fifty hosts (Farr et al., 1989). The physical process of infection and colonization have been studied in depth in several *Colletotrichum* species (Bailey et al., 1992). However, like all fungi, little information has been obtained to explain the genetic or biochemical basis of host colonization and expression of symbiotic lifestyle. *Colletotrichum* species have several attributes which facilitate their use as experimental systems. Mycelial cells and conidia are mononucleate haploids which can be easily mutated and cultured on defined media (Chilton and Wheeler, 1948; Tu, 1985). In addition, several species are amenable to genetic segregation analysis, molecular biological manipulations, and *in vitro* plant colonization and pathogenicity analysis.

C. magna was chosen as the experimental system to study symbiotic lifestyles because it has a heterothallic mating system (Jenkins Jr. and Winstead, 1964; Winstead et al., 1966), is easily mutated (Freeman and Rodriguez, 1992),

transformed (Redman and Rodriguez, 1994; Redman et al., 1999b), and rapidly assayed for plant colonization and pathogenicity in growth chambers (Freeman and Rodriguez, 1992). *C. magna* has a wide host range among different genera from the plant family *Cucurbitaceae* which allowed us to address questions concerning symbiotic lifestyles in several plant species. Over the last 7 years, we have developed a model system to study symbiotic interactions between the filamentous fungus *C. magna* and cucurbit plant hosts (Freeman and Rodriguez, 1992; Freeman and Rodriguez, 1993; Prusky et al., 1994; Redman and Rodriguez, 1994; Redman et al., 1999a; Redman et al., 1999b; Rodriguez and Redman, 2000). The system is based on three observations described in detail below: 1) mutagenesis with uv light and gene disruption converted a pathogenic isolate into mutualists and commensalists, 2) mutualism is based on the ability of the symbionts to confer disease resistance against pathogenic isolates of *Colletotrichum* and *Fusarium*, and 3) disease resistance is correlated to a decrease in the time of activation of host defense systems after exposure to virulent pathogens.

Methodologies and materials

UV and REMI mutagenesis for isolation of nonpathogenic mutants

UV-mutagenesis was conducted on wildtype isolate race 1/2 of *F. o. melonis* and on wildtype isolate FON1 of race 2 of *F. o. niveum* using the previously described method which resulted in the isolation of path-1 of *C. magna* (Freeman and Rodriguez, 1992). Approximately 800 survivors of UV-mutagenesis were screened for pathogenicity on a set of differential cucurbit cultivars. Of these, isolate 4/4 of *F. o. melonis* was completely nonpathogenic and did not cause any disease symptoms using the dip-inoculation screening method (Freeman and Rodriguez, 1993). Four nonpathogenic isolates of *F. o. niveum* were also isolated but were not characterized in this study.

A molecular genetic approach was also taken to isolate nonpathogenic mutants of *C. magna* by restriction enzyme-mediated integration (REMI) with the pHA1.3 plasmid linearized with *Hind* III and transformed into *C. magna* isolate L2.5 in the presence of *Hind* III as described previously (Redman and Rodriguez, 1994).

Plant inoculation and colonization assays

Four physiological races of *F. o. melonis* exist worldwide with race 1/2 being the most virulent race without a resistant cultivar available. Path-1 was assessed for the control of such a *Fusarium* isolate. Seedlings of Ein-Dor cultivar were colonized by path-1 over periods ranging from 1-7 days by dipping in a suspension of 2×10^6 conidia/ml and thereafter exposing to *Fusarium* conidia at concentrations ranging between 10^5 to 10^7 conidia per ml. Conidia of path-1 were either washed or used with media debris. Media was detrimental to plant health and caused mortality of seedlings. A number of commercial cucurbit cultivars were dip-inoculated with path-1 (2×10^6 conidia/ml) over a 10-day period and percent colonization of different tissues were assessed. Plant parts (from roots to cotyledons) were surface sterilized and plated thereafter to determine colonization percentage (see Table 1). Microscopic sections were prepared to determine colonization of the different isolates used.

Biochemical response of plants

The biochemical response of plants to path-1, wildtype *C. magna* L2.5, and cross protection conditions was assessed by analyzing the activity of peroxidase, lignin deposition, and the production of salicylic acid. This was analyzed also in resistant and susceptible watermelon and cucumber plants.

Results

Colonization of various cultivars by path-1 of *C. magna* and 4/4 of *F. o. melonis*

It was found that a differential response of cultivars to path-1 colonization occurred. The melon cultivars Ein-Dor, Hemed and 84, were colonized primarily around the lower stem, crown region with low levels in the upper plant parts. In contrast, the Haon melon and Malali watermelon cultivars were heavily colonized throughout the whole plant. The fungus was recovered at high percentages of 70-85% from cotyledons of the respective cultivars, after one week of continuous dip-inoculation (Table 1).

Table 1. Colonization of plant tissue by path-1.

Cultivar	Inoculation period	Infected plant parts (%)				
	Days	R.	Cr.	M.	U.	Cot.
Ein-Dor (melon)	2	-	52	14	0	-
	4	-	89	44	0	-
	7	-	100	38	0	-
	10	-	100	25	0	-
Hemed (melon)	2	-	60	0	0	-
	4	-	67	0	0	-
	7	-	73	0	0	-
	10	-	53	0	0	-
84 (melon)	2	-	74	20	0	-
	4	-	73	13	6	-
	7	-	60	13	0	-
	10	-	90	0	0	-
Haon (melon)	2	15	100	100	73	-
	5	27	100	100	100	-
	7	71	100	100	92	85
Malali	2	80	100	100	100	-
(w. melon)	5	92	100	100	100	-
	7	87	100	100	100	70

R. = roots; Cr. = crown; M. = middle stem; U. = upper stem; Cot. = cotyledon.

Nonpathogenic mutant isolate 4/4 of *F. o. melonis* colonized a variety of melon cultivars. In Ein Dor cultivar, the optimal concentration of 5×10^6 conidia/ml was required for colonization of between 30 to 50% of lower stem following 7 days continuous dip inoculation. Other concentrations for different time courses yielded reduced colonization percentages. However, isolate 4/4 colonized watermelon seedlings at varying degrees either applied as a continuous dip or mixed into soil (Table 2). Optimal colonization appeared after a dip of 96 hours.

Table 2. Percent colonization of watermelon seedlings (cv. Odem and Malali) by isolate 4/4, mutant of *F. o. melonis* and wildtype *F. o. niveum*, (FON) race 2

Treatment	Isolate				Hours in continuous dip ¹		
	24	48	72	96	120	144	216
Whole plants dipped							
Malali FON	68	58	73	100	100	100	100
Malali 4/4	0	55	28	20	20	38	35
Odem FON	72	66	76	82	100	100	100
Odem 4/4	22	12	16	36	36	22	22
Whole plants in soil							
Odem 4/4	72	82	90	93	100	100	100
Odem FON	0	0	14	18	2	2	-

¹ Plants were dipped continuously in suspensions of 5×10^6 conidia/ml.

Microscopic analysis of plants

Microscopic analysis indicated that path-1 infects and colonizes plants without any indication of a physical response by the plants, even at the penetration sites. The response to the wildtype *C. magna* isolate is very different as the plants respond by deposition of material to wall off the pathogen but only after the pathogen has infected and colonization has begun. Plants colonized with path-1 reacted very strongly to wildtype challenge and appear to completely wall off the pathogen in the first infected cell.

Control of *Fusarium oxysporum* f. sp. *melonis* by a nonpathogenic mutant isolate 4/4 of *F. o. melonis*

In cross-protection experiments with melon cultivars no significant differences were observed between 4/4 colonized challenged plants compared with challenged plants alone. However, isolate 4/4 (originally from melon) was able to reduce wildtype mortality of watermelon seedlings (cv. Odem and Malali) caused by *F. o. niveum* race 2 (Table 2). Colonization percent corresponded to percent of protection. This was observed when at 4 days following optimal colonization, percent mortality

of protected seedlings was most reduced as opposed to lower percent colonization resulting in higher seedling mortality (Tables 2 & 3).

Table 3. Protection of watermelon seedlings (cv. Odem) by nonpathogenic isolate 4/4 of *F. o. melonis* (1.5×10^6 conidia/ml)

Treatment	Plant Mortality (%)			
	Exp #1	Exp #2	Exp #3	
Water		0	0	0
Isolate 4/4	0	0	0	
wt FON ¹ (Inoculated after 4 days)	100	76	100	
wt FON ¹ (Inoculated after 7 days)		88	96	
Isolate 4/4 (4 days) + wt FON		43	47	45
Isolate 4/4 (7 days) + wt FON			60	76

¹FON = Wildtype isolate of *F. o. niveum*, race 2.

Biochemical response of plants

Path-1, non-pathogenic mutant of *C. magna*, was previously shown to protect watermelon and cucumber seedlings from anthracnose disease elicited by wildtype *C. magna*. Disease protection was observed in stems of path-1 colonized cucurbits but not in cotyledons, indicating path-1 conferred either tissue specific and/or localized protection. Plant biochemical indicators of a localized and systemic (peroxidase, phenylalanine ammonia-lyase, lignin and salicylic acid, respectively) “plant defense” response were investigated in anthracnose resistant and susceptible cultivars of cucurbit seedlings exposed to four treatments: 1) water (control), 2) path-1 conidia; 3) wildtype conidia; and 4) challenge conditions (inoculation into path-1 conidia for 48 hours followed by exposure to wildtype conidia). In susceptible plants, the response to path-1 was not significantly different from water controls. The susceptible plants responded strongly to the wildtype isolate but the response did not occur until 4 days after inoculation followed by plant death on day 5. Path-1-colonized susceptible plants responded very quickly and strongly to challenge by L2.5. All biochemical markers were expressed to high levels within 24 hours of challenge and no plant death occurred. The resistant plants responded quickly and strongly to L2.5 as anticipated. Collectively, these analyses indicated that disease protection in path-1 colonized plants was correlated to the ability of

these plants to mount a defense response more rapidly and to equal or greater levels than plants exposed to wildtype *C. magna* alone.

Isolation of REMI mutants and conversion of symbiotic lifestyle by gene disruption:

Over 14,000 REMI transformants were induced with the hygromycin resistance vector pHA1.3 and 176 found to colonize hosts and express non-pathogenic phenotypes (Redman et al., 1999b). All 176 non-pathogenic REMI mutants were indistinguishable from the wildtype with regard to sporulation, adhesion (Sela-Buurlage et al., 1992), germination, appressoria formation, extracellular enzyme production, and growth rates (Redman et al., 1999b). More importantly, the REMI mutants expressed different non-pathogenic symbiotic lifestyles based on the ability to confer disease resistance against *C. magna* and *C. orbiculare* to watermelon plants. The mutants represented three non-pathogenic lifestyles: A - colonized and protected 85 - 100% of the plants against virulent fungi, B - colonized and protected 10 - 65% of the plants against virulent fungi, and C - colonized but did not protect plants against virulent fungi. The integrated vector and flanking genomic DNA sequences in REMI mutant R1 (REMI A) were cloned by digesting genomic DNA from R1 with the restriction enzyme *Xho*I which does not have a recognition site in the pHA1.3 vector, and the resulting product, designated pGMR1, was approximately 11 kb (Redman et al., 1999b). Wildtype *C. magna* was transformed with pGMR1 inducing gene disruption and resulting in same phenotype (A): non-pathogenic and colonized host tissues (Redman et al. 1999b).

Discussion

The *C. magna*-path-1 and *Fusarium*-4/4 mutants did not cause disease symptoms on any cucurbit species but retained their ability to infect and colonize host tissues. In addition to their non-pathogenic endophytic phenotype, we observed that plants colonized with path-1 were protected 100% and 85% against disease caused by virulent isolates of *C. magna* or *Fusarium oxysporum*, respectively (Freeman and Rodriguez, 1993). These pathogens have different mechanisms of pathogenesis which suggested that the protection was not based on the physical occlusion of the virulent fungi by path-1 (Freeman and Rodriguez, 1993). *In vitro*

assays indicated that path-1 was not producing chemical inhibitors of the virulent fungi (Freeman and Rodriguez, 1993). Recently, we demonstrated that path-1 colonized watermelon plants were protected 90 - 100% against disease caused by *C. orbiculare* (Redman et al., 1998). Collectively, these data indicated that the path-1 mutation converted a virulent pathogen to a nonpathogenic endophytic mutualist.

The 4/4 isolate of *F. o. melonis* was capable of colonizing melon and watermelon seedlings without causing disease whereas the *F. o. melonis* wildtype isolate did not colonize watermelon and did not cause disease. Although isolate 4/4 did not protect against the wildtype pathogen in melon, protection was achieved against the *F. o. niveum* pathogen specific to watermelon. Thus it appears that pathogenicity genes in *Fusarium* may be linked to genes involved in colonization.

The ability of path-1 to grow through host tissue without eliciting disease may be dependent on either suppression or avoidance of the host defense systems. Since the path-1 colonized plants are resistant to virulent wild type fungi (*C. magna*, *C. orbiculare*, and *F. oxysporum*) it is unlikely that path-1 is suppressing the host defense systems. We have performed biochemical studies which indicate that path-1 avoids activation of host defenses and that path-1 colonized plants, challenged with virulent isolates, activate host defenses more quickly than control plants (Redman et al., 1998). These results were determined by monitoring the activity of peroxidase and the deposition of lignin in path-1 colonized and control plants.

To determine the genetic complexity of mutations capable of changing the symbiotic lifestyle of *C. magna*, we generated non-pathogenic mutants by gene disruption using pGMR1 containing the "tagged" putative pathogenicity genes. Gene disruptions in *Colletotrichum* species have been accomplished by homologous integration of transformation vectors containing either disrupted or truncated genes (Bowen et al., 1995; Hwang et al., 1995b). These results indicate that gene disruption events can occur at a high frequency in *C. magna*.

Scientific implications

Colletotrichum has become a valuable experimental system to study genetic, molecular, and biochemical aspects of plant-fungal symbiotic associations. Current

technologies have allowed us to isolate a variety of nonpathogenic mutants by UV and gene disruption techniques which expressed endophytic phenotypes. Two of these mutants (REMI phenotypes A & C) have identified genes that are responsible for changing the symbiotic lifestyle of *C. magna* from pathogenic to mutualistic or commensalistic, respectively. Although these mutations do not indicate the evolutionary direction of these different symbiotic lifestyles (ie. did pathogens evolve from commensal or mutualists), they do indicate that lifestyle conversions may occur with genetic simplicity (single gene disruptions). The biochemical ramifications of the gene disruptions resulting in lifestyle conversions may be very significant. If the mutations are pleiotrophic, the impacts may not be easily observed and may not affect the ability of these fungi to perform as commensals or mutualists. As more of the genes responsible for the non-pathogenic REMI mutants are cloned and analyzed the genetic complexity of these phenotypes will be defined. More importantly, this system will lead to an understanding of the genetic basis of commensalistic, mutualistic, and pathogenic lifestyles. Once the genetic basis of these symbiotic lifestyles is defined, the role of fungi in ecosystem structure and dynamics will be better understood.

Potential impact on agriculture

The nonpathogenic mutants isolated and “created” are potentially novel biocontrol agents. To date no biocontrol agents have been used that were derived from wildtype pathogenic isolates. For example, in *Fusarium*, only specific pathogens used in hosts that are unaffected by these organisms as well as saprophytes have been utilized for biocontrol. These mutants therefore may compete better than their “parents” when applied in the correct manner.

Besides the potential use as biocontrol agents, the non-pathogenic mutants will enable a better understanding of the genes involved in pathogenicity.

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