**BARD Final Scientific Report
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Date of Submission of the report: February, 2009

BARD Project Number: US-3774-05C

Project Title: Gene Expression Patterns in Plants Colonized with Pathogenic and Non-pathogenic Gene Disruption Mutants of *Colletotrichum*.

Investigators

Principal Investigator (PI):

Russell J. Rodriguez

Co-Principal Investigator (Co-PI):

Stanley Freeman

Institutions

Univ. of Washington, WA, USA

ARO, Volcani Center, Israel

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Disease, fungi, gene expression, microarray, mutualism, pathogenicity genes, plant protection, symbiosis, symbiotic lifestyle

Abbreviations commonly used in the report, in alphabetical order:

DNA - deoxyribonucleic acid

mRNA - messenger ribonucleic acid

SSH - suppressive subtractive hybridization

Budget: IS: \$145,000

US: \$155,000

Total: \$300,000

Signature

Principal Investigator

Signature

Authorizing Official, Principal Institution

**Lynne Chronister
Executive Director
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*Grant & Contract Administrator
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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)		4	2	6
Submitted, in review, in preparation	2	2	1	5
Invited review papers		1		1
Book chapters		2		2
Books				
Master theses				
Ph.D. theses			1	1
Abstracts		8	4	12
Not refereed (proceedings, reports, etc.)			3	3

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Dr. Claire Woodward (USA) _____

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	-	1	1	2
Longer Visits (Sabbaticals)	-	-	-	-

Description of Cooperation:

The US and Israeli cooperators have met twice to discuss experimental results and to plan additional experiments. We have exchanged data for sequence and bioinformatics analysis, and coordinated experiments to optimize data generation and analysis. These meetings have been fruitful and allowed us to coordinate efforts and submit a continuation proposal to BARD.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted	-	-	-	-
Issued (allowed)	-	-	-	-
Licensed	-	-	-	-

Abstract

Fungal plant pathogens are responsible for extensive annual crop and revenue losses throughout the world. To better understand why fungi cause diseases, we performed gene-disruption mutagenesis on several pathogenic *Colletotrichum* species and demonstrated that pathogenic isolates can be converted to symbionts expressing non-pathogenic lifestyles. One group of non-pathogenic mutants confer disease protection against pathogenic species of *Colletotrichum*, *Fusarium* and *Phytophthora*; drought tolerance; and growth enhancement to host plants. These mutants have been defined as mutualists and disease resistance correlates to a decrease in the time required for hosts to activate defense systems when exposed to virulent fungi. A second group of non-pathogenic mutants did not confer disease resistance and were classified as commensals. In addition, we have demonstrated that wildtype pathogenic *Colletotrichum* species can express non-pathogenic lifestyles, including mutualism, on plants they colonize asymptotically.

We have been using wildtype and isogenic gene disruption mutants to characterize gene expression patterns in plants colonized with a pathogen, mutualist or commensal. The US group is contrasting genes expressed during colonization by mutualistic and commensal mutants of *C. magna* and a pathogenic wildtype *C. coccodes* on tomato. The Israeli group is characterizing genes expressed during asymptomatic colonization of tomato by wildtype *C. acutatum* and a non-pathogenic mutant. To accomplish this we have been utilizing suppressive subtraction hybridization, microarray and sequencing strategies.

The expected contribution of this research to agriculture in the US and Israel is: 1) understanding how pathogens colonize certain hosts asymptotically will shed light on the ecology of plant pathogens which has been described as a fundamental deficiency in plant pathology; 2) identifying genes involved in symbiotically conferred disease resistance will help explain why and how pathogens cause disease, and may identify new candidate targets for developing genetically modified disease resistant crop plants.

Achievements

During the first two years of this grant (2006 & 2007), both the US and IS labs generated a series of cDNA pools from tomato plants colonized with virulent wildtype and nonpathogenic mutants of *Colletotrichum* species to construct a series of Suppressive-Subtraction-Hybridization (SSH) libraries. The US lab has focused on tomato plants (cv. Tiger-like) colonized with *C. magna* isolates and the IS lab has also focused on tomato plants (cv. Tiger-like) colonized with *C. acutatum* isolates. The tomato libraries in the US are designed to compare the gene expression patterns in plants colonized with a mutualist or a commensal - both of which are pathogenic on cucurbits. In Israel, the tomato libraries are designed to compare gene expression patterns in plants colonized by wildtype and nonpathogenic *C. acutatum* isolates from strawberry, both nonpathogenic on tomato. These isolates were generated as part of a previous BARD grant and are referred to as non-restricted pathogens. In 2007 we completed the sequence analysis of the SSH libraries and began generating replicate libraries for comparison. In 2008 we completed the sequence analysis of the replicate libraries and began the bioinformatic analysis of the cDNA sequences from the SSH libraries representing *C. magna* and *C. acutatum* expressing either a mutualist, commensal or pathogenic lifestyle. Initial results are very interesting and suggest that the non-pathogenic isolates are recognized by host plants and stimulate a number of host genes involved in different aspects of photosynthesis, catabolism and anabolism. Interestingly, we did not identify any recognizable fungal genes from the SSH analysis. We surmise that the lack of fungal genes reflects the extremely low abundance of fungal DNA versus plant DNA so focused the remainder of our time on the isolated plant genes. We have also begun a series of microarray analyses to confirm SSH results and identify cellular pathways involved in symbiotic communication.

Over the next several months both laboratories will complete bioinformatics analyses to identify target genes for additional studies. All differentially expressed genes will be assessed for conservation across phylogenetically related and distant host species. In addition, candidate up-regulated genes (stress and ROS such as catalase, peroxidase, PR protein 5 and superoxide dismutase) will be used for gene expression patterns throughout the colonization process. The experiments are very lengthy to set-up, run and analyze, and we are very content with the progress made over the last three years.

Significance of main scientific achievements:

The results generated so far indicate that all of the fungi tested in this study communicate with plant hosts resulting in different gene expression patterns. We conclude that the fungi are recognized by the host regardless of the lifestyle (mutualism, commensalisms, parasitism) being expressed. However, plant gene expression patterns differ depending on the fungal species and the lifestyle expressed by isolates of one fungal species. Therefore, we hypothesize that the expression of specific plant genes reflect the type of symbiosis and that these "symbiotic indicator" genes may be used to predict optimal mutualistic interactions between plants hosts and fungi that confer stress tolerance. Eventually, this type of information will be incorporated into breeding programs to optimize plant fitness.

Agricultural impacts of the research findings

The expected long term impact of this research to agriculture in the US and Israel demonstrates that host-specific mutualists may be utilized in the various crops to confer (1) disease resistance to reduce dependence on chemical fungicides; (2) drought tolerance to reduce water consumption for irrigation; and (3) growth enhancement to increase yields.

Details of cooperation

The US and IS researchers have a very healthy cooperation on this project. The US lab has constructed the gene disruption vectors that were used to generate the mutants. The species chosen for this work have different pathologies and host ranges making them optimal candidates for determining if this novel strategy to identify symbiotic genes associated with mutualism, commensalism and pathogenesis.

The US and IS labs frequently communicate about ongoing experiments and freely share data from previous research. During 2007, SSH libraries constructed in Israel were sent to the US for sequence analysis. The corresponding data was returned to Israel for bioinformatic analysis. At the same time SSH libraries were constructed and analyzed in the US lab so that comparative analyses could be performed. In March 2007, Freeman traveled to Seattle to meet with Rodriguez to discuss research results and to plan experiments for 2007/8. In 2008, the SSH analyses were completed and a list of genes identified that were differentially expressed between plants colonized with different fungi. The complexity of the results warranted expanding the

effort into microarray analysis to confirm the SSH results and to develop rtPCR primers for genes of interest. The investigators will meet again in March of 2009 to discuss the microarray data and outline manuscripts for publication in peer-reviewed journals. Manuscripts based on bioinformatics analyses will be co-authored by the US and IS researchers.

List of publications:

- Horowitz, S., Freeman, S., Zveibil, A., and Yarden, O. 2006. A defect in nir1, a nirA-like transcription factor, confers morphological abnormalities and loss of pathogenicity in *Colletotrichum acutatum*. *Mol. Plant Pathol.* 7:341-354.
- Horowitz Brown, S., Yarden, O., Gollop, N., Chen, S., Zveibil, A., Belausov, E., and Freeman, S. 2008. Differential protein expression in *Colletotrichum acutatum*: changes associated with reactive oxygen species and nitrogen starvation implicated in pathogenicity on strawberry. *Mol. Plant Pathol.* 9:171-190.
- Rodriguez RJ, Woodward C, Kim YO, Redman RS. 2009. Habitat-Adapted Symbiosis as a Defense against Abiotic and Biotic Stresses. In *Habitat-Adapted Symbiosis as a Defense against Abiotic and Biotic Stresses*, Eds. M. Torres and J. White, New York, Boca Raton, FL. Taylor & Francis Group, pg 333-344, *In Press*.
- Rodriguez RJ, White JFJ, Arnold AE, Redman RS. 2008. Fungal endophytes: diversity and functional roles. *Tansley Review, New Phytologist*. *In Press*.
- Kim YO, Rodriguez RJ, Lee EJ, Redman RS. 2008. *Phytolacca americana* from contaminated and noncontaminated soils of South Korea: effects of elevated temperature, CO² and simulated acid rain on plant growth response. *Journal of Chemical Ecology*, *In Press*.
- Rodriguez R.J. and Redman R.S. 2008. More Than 400 Million Years Of Evolution And Some Plants Still Can't Make It On Their Own: Plant Stress Tolerance Via Fungal Symbiosis. *Journal of Experimental Botany*, **59**:1109-1114.
- Rodriguez R.J., Henson J., Van Volkenburgh E., Hoy M., Wright L., Beckwith F., Kim Y., Redman R.S. 2008. Stress Tolerance in Plants via Habitat-Adapted Symbiosis. *ISME-Nature*, **2**:404-416.
- Rodriguez, R.J. and Redman R.S. 2005. Balancing the generation and elimination of reactive oxygen species. Commentary in the *Proceeding of the National Academy of Sciences*, **102**:3175-3176.
- Henson, J.M., Redman R.S., Rodriguez, R.J. and Stout R. 2005. Fungi in Yellowstone's geothermal soils and plants. *Yellowstone Science*, **13**:25-30.
- Rodriguez, R.J., Redman R.S., and Henson, J.M. 2005. Symbiotic Lifestyle Expression by Fungal Endophytes and the Adaptation of Plants to Stress: Unraveling the Complexities of Intimacy. In *The Fungal Community: Its Organization And Role In The Ecosystem*, Ed. J. Dighton, P Oudemans & J. White. Boca Raton, FL. Taylor & Francis/CRC Press, pg 683-96.