Project Report - March, 2010

Project Title: Genomic sequencing of biocontrol strain EB92-1 and identification of elicitor(s) of effective defense in *Vitis vinifera* against Pierce's Disease.

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Reporting Period: The results reported here are from work conducted from 11/13/09- 2/28/10

List of Objectives and Activities Accomplished:

Objective 1: obtain nearly the complete EB92-1 genome DNA sequence (1 year).

This work was not funded until 11/13/09. In the first three months, we have obtained * a total 2,478,233 bps of EB92-1 sequence, with a breakdown as follows:

* Average Length: 11,801 bps (Min: 106 bps, Max: 149,096 bps)
* Average GC Content: 53% (Min: 30%, Max: 70%)
* Gene Coding Percentage: 81%

Predicted gene elements:

* 2387 genes coding for mRNA (protein coding ORFs)
* 48 genes coding for tRNA
* 3 genes coding for rRNA
* 0 repetitive elements

By far the majority of the primary Blast hits were to Temecula; based on the size of the Temecula genome (2,519,802 nt), this draft EB92.1 genome is ca. 98% complete. Comparative analyses of Temecula vs. EB92.1 using Mauve revealed that EB92.1 is highly similar to Temecula and the gene order exhibits a very high level of synteny.

Objective 2: compare EB92-1 with Temecula and identify all unique ORFs and differences, ranking the top 40 candidate ORFs for evaluation as elicitors (1 month).

No additional hemolysins or colicins were found to date in the draft EB92.1 genome that were not found in Temecula. More importantly, no additional hemolysins or colicins were present in Temecula that were not found in EB92.1. The fact that the complete repertoir of known Temecula Type I effectors were found, with 100% identity, in EB92.1 (which does not cause PD), means that PD symptoms per se are not likely caused by these effectors, although an essential role in host colonization or adaptation is still indicated (Flores-Cruz et al. 2009).

Several cell wall degrading enzymes with Type II secretion leader sequences that are found in Temecula are clearly missing in EB92.1; these were very recently cloned and expressed and are being evaluated as potential pathogenicity factors in Temecula.

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Objective 3: evaluate two defense response assays designed to test the hypothesis that EB92-1 produces an elicitor that Temecula does not (9 months)

Not yet attempted (2nd year goal).

Objective 4) perform "avirulence" assay screens using up to 40 Temecula transconjugants carrying up to 40 candidate ORFs identified from the EB92-1 genome in Objective 2 (1 year).

Not yet attempted (2nd year goal).

Objective 5) perform defense response assay screens to confirm any suspected elicitors identified in Objective 4 and identify any elicitors that may be polygenic in nature (ie., LPS or cell fractions) using the best of 3 assays determined by Objective 3 (1 year).

Candidate genes encoding cell wall degrading enzymes identified from the EB92-1 sequence in Objective 2 as present in Temecula but missing in EB92-1 have been cloned into the pET27B expression system (Novagen). Expression assays are underway.

IV. Summary of major research accomplishments

A draft EB92.1 genome that currently contains 2,478,233 bps of genomic sequence in 210 contigs has been created. The average contig length is currently 11,801 bps (Min: 106 bps, Max: 149,096 bps). By far the majority of the primary Blast hits are to Temecula; based on the size of the Temecula genome (2,519,802 nt), this draft EB92.1 genome is ca. 98% complete. By far the majority of the primary Blast hits were to Temecula; based on the size of the Temecula genome (2,519,802 nt), this draft EB92.1 genome is ca. 98% complete. Comparative analyses of Temecula vs. EB92.1 using Mauve revealed that EB92.1 is highly similar to Temecula and the gene order exhibits a very high level of synteny.

No additional Type I effectors (hemolysins or colicins) were found to date in the draft EB92.1 genome that were not found in Temecula. The fact that the complete repertoir of known Temecula Type I effectors were found, with 100% identity, in EB92.1 (which does not cause PD), means that PD symptoms per se are not likely caused by these effectors, although an essential role in host colonization or adaptation is still indicated. Several cell wall degrading enzymes with Type II secretion leader sequences that are found in Temecula are clearly missing in EB92.1; these were very recently cloned and expressed and are being evaluated as potential pathogenicity factors in Temecula.

V. Publications or reports resulting from the project:

Gabriel, D. W. and D.L. Hopkins. 2009. Role of Type I Secretion in Pierce's Disease. Symposium Proceedings of the 2009 Annual Pierce's Disease Meeting, December 9-11, 2009, pp 86-91.

Flores-Cruz, Z, Reddy, S, Hopkins, D.L., and D. W. Gabriel. 2009. Potential offensive role of the Type I Secretion System in *Xylella fastidiosa. Poster* PS2-50, presented at the 2009 Annual Pierce's Disease Meeting, December 9-11, 2009.

VI. Presentations on research

Flores-Cruz, Z, Reddy, S, Hopkins, D.L., and D. W. Gabriel. 2009. Potential offensive role of the Type I Secretion System in *Xylella fastidiosa*. Poster presentation at the IS-MPMI meetings, July 19-23, Quebec, Canada.

Gabriel, D. W. and D.L. Hopkins. 2009. Role of Type I Secretion in Pierce's Disease. Symposium Proceedings of the 2009 Annual Pierce's Disease Meeting, December 9-11, 2009, pp 86-91.

Flores-Cruz, Z, Reddy, S, Hopkins, D.L., and D. W. Gabriel. 2009. Potential offensive role of the Type I Secretion System in *Xylella fastidiosa. Poster* PS2-50, presented at the 2009 Annual Pierce's Disease Meeting, December 9-11, 2009.

VII. Research relevance statement

If pathogenicity genes are found in Temecula that are not found in biological control strain EB92-1, and if these genes can be demonstrated to be primarily responsible for generating PD symptoms, then methods to interfere with the mechanism of action of such genes may be found. Similarly, if genes are found in EB92-1 that suggest a mechanism for the biological control of PD strains such as Temecula, these may be directly used in attempts to control PD or may suggest methods previously not considered to control PD.

VIII. Lay summary of current year's results

To date, we have almost completed the genomic DNA sequence of the biological control strain EB92-1, which can stably infected Vitis vinifera grapes, but without causing PD or other disease symptoms. This is allowing rapid comparisons with the Xf strain Temecula (PD) genome, resulting in the identification of several genes that may be needed by Temecula to cause PD. Identification of required pathogenicity genes may suggest novel methods for controlling PD.

IX. Status of funds

Approximately $20,000 has been spent on genomic sequencing of EB92.1

X. Summary and status of intellectual property produced during this research project

None generated to date.