**Renewal Progress Report for CDFA Agreement Number 14-0142-SA**

**Title of Project**

Exploiting a chitinase to suppress *Xylella fastidiosa* colonization of plants and insects

**Principal Investigator**

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**Time Period Covered by the Report**

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**Introduction**

The goal of this new project is to determine which protein(s) interact with a chitinase (ChiA) we identified in *X. fastidiosa*. A *chiA* mutant strain is deficient in chitin degradation, insect and plant colonization, as well as plant-to-plant vector transmission. The gamut of biological activities associated with ChiA make it a desirable target to limit *X. fastidiosa* colonization of plants and vectors, as well as Pierce’s disease spread. However, although active, we did not identify a chitin-binding domain in the sequence of *chia* using bioinformatics tools, and we showed experimentally that ChiA is not active without the presence of other *X. fastidiosa* proteins. The goal of this project is to identify these partners and the role of ChiA in plants so that disease control strategies can be developed exploiting ChiA by suppressing its biological roles.

**List of Objectives – as in the approved research proposal**

i) to identify *X. fastidiosa* proteins or protein complexes that bind to ChiA and are required for its chitinolytic activity.

ii) to screen potential substrates cleaved by ChiA.

iii) to functionally demonstrate the role of ChiA partners during insect and plant colonization.

**Description of activities**

Prior to summarizing current activities, a note on personnel issues must be made, as it has impacted proposed activities for the first semester of this work. The postdoctoral researcher hired to work on this project left for a position a couple of months after the project initiated last summer. A technician was hired immediately upon notification so that the researcher could train the technician for a few weeks on the essentials of the project, so that basic expertise on *X. fastidiosa* and specific aspects of this project were not lost prior to the arrival of a new researcher. In addition, and effort was made to finalize all details of previous work on ChiA done by this researcher prior to his departure, which we are soon submitting for publication. This has allowed the project to move forward but on a much slower pace; recruitment of a new researcher has been ongoing and we hope that the position will be filled in the very near future. The technician will then overlap with the new hire for some time so that the project moves faster at that stage.

Current work has focused on the identification of chitin-binding proteins associated with ChiA. To identify the ChiA partner *Xylella fastidiosa* (Temecula) was grown on solid XFM media supplemented with either galacturonic acid (GA) or colloidal chitin (0.1g of either) in order to simulate a plant or insect environment and induce phenotypic changes in the bacteria (Killiny and Almeida 2009, Killiny et al. 2010). Cells were scraped after 10 days and resuspended (PBS initially, then TE; both have worked well), with 1μM PMSF added to inhibit protease activity (Killiny et al. 2010). Cells were lysed via 5 cycles of freeze-thaw, and concentration was determined according to Bradford. An IMAC Ni resin column (BioRad) was used in an attempt to isolate ChiA binding partners. After purified ChiA (produced by our group following standard protocols, from recombinantly expressed *E. coli* cell) was bound to the column, whole cell lysate was applied and allowed to incubate for 30 minutes. Working on the assumption that the proteins of interest would remain bound to the ChiA and elute with it, the column was then washed with increasing concentrations of imidazole in PBS. The fractions were then run in SDS-PAGE, which denatures and should therefore cause protein complexes to disassociate, against a purified ChiA control, and stained with Coomassie blue. No proteins were found to elute with the ChiA fractions, the experiment was repeated several times and, for now, we have decided to not pursue this approach any longer.

Alternatively, more recently a solution of 4g colloidal chitin from shrimp shells (Sigma) was prepared. Five milliliters of the solution was allowed to settle in the column, which yielded a column volume of approximately 1ml. Ten volumes of 2M NaCl were washed through the column prior to loading, and then the column was equilibrated with the aforementioned solution. Whole-cell lysate from Temecula grown on XFM-GA was applied to the column and allowed to incubate for 30 minutes. Increasing concentrations (0.025M, 0.05M, 0.1M, 0.25M, 0.5M, and 1M) of methyl-alpha-D-glucopyranose were used to elute proteins bound to the column. Fractions were run on SDS-PAGE and stained with Coomassie blue. Many bands appeared in the early washes, indicating non-specificity for chitin. One prominent band appears in all subsequent fractions. There should be more bands in the later fractions, but we expect those to have lower concentrations and silver staining will now be used. This approach is simpler than others we hope to run with a postdoctoral researcher, but has now yielded satisfactory results and we expect to soon work on repeating this work so that samples can be sent out for identification of promising bands in late elutions.

One last approach has been tested. This approach to determine ChiA binding partners involved overlaying an agarose gel containing 4-MU(GlcNAc)3 (Sigma) on a native PAGE gel containing whole cell lysate from both chitin- and GA-grown cells incubated with purified ChiA. As 4-MU(GlcNAc)3 only fluoresces when the MU subunit has been cleaved, fluorescence indicates the presence of a functional chitinase (Killiny et al. 2010). Initial assays suggest that optimization of pH in native gels must be performed. No detectable ChiA migrated in the native PAGE, but aliquots from the same sample displayed a positive band in the SDS-PAGE, the pH of the gel may be incompatible with the isoelectric point of the protein. An effort is being made to optimize these experimental conditions. The detectable band in native gel will be cut out and prepared for sequencing following standard protocols.

In summary, three approaches were tested. The first one aimed at identifying proteins binding to ChiA failed and will not be continued, at least for now. Another had promising results and will lead to the identification of chitin-binding proteins in the near future. The last approach should identify a protein that is required by ChiA for its activity in gel, but the protocol requires some optimization. Therefore, despite our personnel problems, the project is successfully moving forward, albeit slowly than originally proposed.

**Publications produced and pending, and presentations made that relate to the funded project**

Publications: a manuscript describing the role of ChiA on the biology of *X. fastidiosa* is currently being finalized and will be submitted for publication soon.

Presentations:

. Exploiting a chitinase to suppress *Xylella fastidiosa* colonization of plants and insects. Pierce's Disease Research Symposium, Sacramento, CA.

. Pierce's disease in winegrapes and olive. 3rd Annual Vineyards & Wineries Continuing Education Class Series, Nov 4, 2014, Napa, CA. Sponsored by UCCE, Farm Bureau and the Napa Ag Commissioner's Office.

. Emerging Vector-borne Diseases; Forum on Microbial Threats, Institute of Medicine, National Academy of Sciences, Washington DC.

**Research relevance statement**

We have shown that ChiA plays a central role in the *X. fastidiosa* transmission cycle. It is also essential for the successful colonization of both plant and insect hosts of *X. fastidiosa*. However, there are several important questions related to the activity of this enzyme. First, it is not clear what substrates it cleaves, especially in plants. Second, ChiA is not active by itself, apparently requiring a partnership with substrate-binding proteins. We propose to characterize ChiA so that we can suppress its activity in both plants and insects. ChiA represents a unique target to control Pierce’s disease because it is required for the colonization of both plants and insects, in addition to being important for vector transmission and spread. Therefore, disruption of its activity should lead to control of *X. fastidiosa* colonization of plants and movement from plant-to-plant.

**Layperson summary of project accomplishments**

This project started in the Summer of 2014 and suffered from the departure of the postdoctoral researcher working on it a few months later. A qualified but new technician was hired on short notice to continue the work until the arrival of the new researcher. The technician has been focusing on the testing and troubleshooting of protocols under the supervision of the PI and our prior researcher. This new project is advancing forward with success, but at a slower pace than proposed.

**Status of funds**

Funds are being used slower than originally proposed. This is due to the problem associated with the sudden departure of the post-doctoral researcher originally working on this project, the search for a new researcher, and hire of a technician in the meantime to continue with vital components of the project. We were fortunate to hire a very qualified technician, who was briefly trained by the previous researcher, is troubleshooting protocols, and will teach our new hire on the essentials of the project. This permit that time is not lost during what could be a prolonged adaptation period to *X. fastidiosa* and specifics of this project by the new hire.

**Summary and status of intellectual property associated with the project**

Not applicable at this stage.

**Literature cited**

Almeida, R.P.P. and Labroussaa, F. 2014. Blocking *Xylella fastidiosa* transmission. Proceedings Pierce’s disease research symposium, December 1-17, Sacramento, p. 202-210.

Killiny, N. and Almeida, R.P.P. 2009. Host structural carbohydrate induces vector transmission of a bacterial plant pathogen. Proceedings of the National Academy of Sciences USA 106: 22416-22420.

Killiny, N., Prado, S.S. and Almeida, R.P.P. 2010. Chitin utilization by the insect-transmitted bacterium *Xylella fastidiosa*. Applied and Environmental Microbiology 76: 6134-6140.

**FUNDING AGENCIES**

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