# CITRUS ADVANCED TECHNOLOGY PROGRAM

**QUARTERLY & FINAL REPORTS:** Control of Citrus Greening, Canker & Emerging Diseases of Citrus

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

## Citrus canker strains survive in biofilms on leaves fruit

Proposal Title

Survival of Xanthomonas citri ssp. citri (Xcc) to estimate risk of citrus canker transmission by infected fruit

| Today's Date | Sponsoring Organization (drop-down) | Category (drop down) |
|---|---|---|
| 7/15/11 | Citrus Research and Development Foundation | Infection Consequences |

**REPORT UPDATE** (500 words; summarize your accomplishments )

Objective 1 (confirm biofilm formation by X. citri subsp. citri (Xcc) in comparison with other bacteria that are well known to form biofilms). In previous work using stable and labile Gfp expressing strains and confocal laser microscopy and a colorimetric assay involving crystal violet staining, aggregation and biofilm formation were confirmed for wide (XccA) and limit host range strains (XccA* and XccAw) of Xcc. Higher aggregation and biofilm formation was demonstrated for Xcc-A than XccA-Aw or XccA-A*. This higher biofilm formation of XccA was shown in vivo and in vitro after propagation on several culture media. However, further evaluation using the media XVM2, a medium commonly used to simulate plant conditions for gene expression analysis, behavior of 306 strain (XccA) and 407 (XccA*) was reversed. Aggregation by 407 strain in XVM2 medium was promoted compared to Xcc-A 306. To determine which component of XVM2 medium is involved in this biofilm response of XccA*, aggregation of strain 407 was screened in LB by omitting each of the components (casein, fructose, $MgSO_4$, $(NH_4)_2SO_4$, NaCl, $CaCl_2$, $FeSO_4$, $KH_2P_4$, $K_2HP_4$). $MgSO_4$ was identified as the component that most affected biofilm formation and bacterial survival. Mg has been associated with flagellar integrity as well as aggregation processes in other bacterial models, whereas, our results reveal that flagella-like structures are related to the enhancement of biofilm formation. Greater flagellation and presence of swarming cells (with high ability for movement) in the XccA strains compared toXccAw demonstrates that flagella-like structures may account for the difference in biofilm formation between these strains. When other compnents were removed from XVM2 medium, less survival or biofilm in strain 407 indicates that biofilm formation process is a complex system related to more than one environmental variable. Influence of $Mg^{+2}$ is being currently studied in order to elucidate a possible role in expression of genes related to biofilm formation as well as flagellation and bacterial motility in Xcc. Differences in biofilm formation and motility among wide and limit host range strains may be at least one of the bases for their difference in virulence. Regarding gene expression analysis new assays confirmed higher presence of the GumD mRNA in biofilm than in bacterial suspension. In contrast FleN, a flagellation regulator, is more highly expressed in bacterial suspension than biofilm in Xcc. FleN has been described as a negative regulator for multiflagelation in other bacterial models and our results point out its possible role in formation of biofilm cells in Xcc.

Objective 2 (biofilm formation under different conditions). In previous assays biofilm disruption was evaluated in vitro after treatments with peroxide, $CuSO_4$, SOPP, NaCl and NaClO at different concentrations by colorimetric assay. $CuSO_4$ and peroxide were not able to disrupt and remove the bacteria from the surface. Experiments with the limited host range strains giving similar results. Viability of these remaining aggregates is being currently evaluated to determine if those compounds, that do not disrupt the biofilm, are still lethal to the bacteria. Biofilm disruption is also being evaluated in vivo. To do so, biofilm formation is induced in leaf dishes on petri plates under controlled conditions in a growth chamber. After 72 hours leaf incubation with XccA, dish leaf surfaces were sprayed with the different bactericide compounds previously tested. In the first assay already performed with high concentration of those compounds, living bacteria were found after NaCl, SOPP and peroxide treatments. New experiments are in progress with a range of concentrations of the bactericides.


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SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.