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)

Identification of potential inhibitors against SecA of Las

Proposal Title

Control of Citrus Huanglongbing by disruption of the transmission of citrus greening pathogen by psyllids

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
1/14/11	Citrus Research and Development Foundation	Unclassified

REPORT UPDATE (500 words; summarize your accomplishments)

Management of citrus Huanglongbing (HLB), which is associated with Candidatus Liberibacter asiaticus, could be achieved by application of antimicrobials and stopping the spread of HLB pathogen. Curing Ca. L. asiaticus infected citrus trees is one attractive goal due to the high value of citrus trees and the high cost of citrus tree removal and replanting. Wide spread of HLB throughout Florida renders curing Ca. L. asiaticus infected citrus trees necessary. Treatment of Ca. L. asiaticus infected citrus could be pursued by applying antimicrobials to infected trees. The most common targets for antimicrobial agents include receptors, proteins and enzymes, DNA, RNA and ribosomal targets. Among them, proteins have become the major target due to their druggable characteristics. In this study, we presented our research on screening small molecule inhibitors against SecA. SecA is one essential component of the Sec machinery which provides a major pathway of protein translocation from the cytosol across or into the cytoplasmic membrane. The Sec pathway was also shown to be required for virulence of Ca. L. asiaticus in our study. SecA is the protein translocase ATPase subunit, which involves in pre-protein translocation across and integration into the cellular membrane in bacteria.
First we filtered the structures based on their physico-chemical properties, e.g. Molecular Weight, H-Bond Donor, H-Bond Acceptor & Rotatable bonds and structurally similar to adenine moiety. Approximately 5000 structures were retrieved from ~5 million structures of commercially available databases. The identified data set was used for virtual screening by molecular docking method. Based on the dock scores we eliminated about 4500 low scored structures and selected ~500 (10%) for further molecular docking & minimization to evaluate the scoring functions (Dock glide scores, Hydrophilic, Hydrophobic e.t.c). Based on scoring functions, structural diversity, and our chemical intuition we have chosen forty structures for biological activity studies against purified SecA protein. For that purpose, SecA of Ca. L. asiaticus was expressed in E.coli using the pE-SUMO vector and purified. The inhibition assays of the 40 compounds against SecA was done as described previously by Denis et al. 1978. The IC50 ranged from 0.3µM to 100µM. Currently, we are testing the antimicrobial activities of the top 10 compounds against Sinorhizobium meliloti, Agrobacterium tumefaciens, and E.coli. In addition, those compounds will serve as leads to develop new compounds.

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Organization Citrus Research and Education Center

Contract Number 337

Project Duration (years) 3 *Year of Project* 1

Total Funds (current year) $114,714.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.