Re-Examining the Role of *Rhodococcus* in Pistachio Bushy Top Syndrome

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INTRODUCTION

This project seeks independent validation of the claim that Pistachio Bushy Top Syndrome (PBTS) is caused by bacteria belonging to the genus *Rhodococcus*, as reported in a scientific article from the group of Dr. Jennifer Randall at New Mexico State University (Stamler et al 2015 Plant Disease 99:1468-1476). The article documents the results of a greenhouse study showing development of PBTS-like symptoms on clonal UCB-1 pistachio trees, following inoculation with *R. fascians* Isolate 2 and/or *R. corynebacterioides* Isolate 1, originally isolated from PBTS-impacted trees. As explained in our original proposal, one of the reasons for a re-examination of this claim involves an inconsistency following a second publication by the same group (Stamler et al 2016 Genome Announcements 4:e00495-16), which presents the genome sequences of *R. fascians* Isolate 2 (renamed *R. fascians* PBTS2) and *R. corynebacterioides* Isolate 1 (renamed *R. corynebacterioides* PBTS1). The genomes were published along that of the model plant-pathogenic bacterium *R. fascians* D188, which harbors a linear plasmid pFiD188 carrying the so-called *fas* locus. It has been established by other researchers that without this locus (or the plasmid), strain D188 does not cause disease. This also is the basis for using *fas* genes as diagnostic markers for rhodococcal pathogenicity. Closer inspection revealed that the published PBTS2 and PBTS1 genomes do not contain a pFiD188-like plasmid or *fas*-like genes, which directly contradicts the Stamler 2015 paper by the same group.

The objectives of the project are as follows: 1) reproduce PBTS symptoms on clonal UCB1 trees by inoculation with *R. fascians* Isolate 2/PBTS2 and/or *R. corynebacterioides* Isolate 1/PBTS1, following the published 2015 Stamler et al protocol; 2) compare the impact of Isolate 2/PBTS2 and Isolate 1/PBTS1 on pistachio to that of other bacteria, including *R. fascians* D188 and its plasmid-cured derivative, and/or a selection of genome-sequenced rhodococcal and non-rhodococcal isolates from pistachio trees in California; 3) test the effect of tree rootstock, tree age, and tree tissue type on symptom formation by *Rhodococcus*; and 4) develop DNA-based methods to profile the microbial communities (including *Rhodococcus*) that associate with naturally or artificially contaminated pistachio trees and tissue types.

RESULTS

Objectives 1, 2, and 3 are dependent on the availability of the original *R. fascians* Isolate 2 and *R. corynebacterioides* Isolate 1. We have requested both isolates from Dr. Randall at New Mexico State University (NMSU) but to this date (December 1, 2017) have not received them. We are currently negotiating with NMSU over the language describing the isolates in a Material Transfer Agreement (MTA) that would allow us to receive the *Rhodococcus* strains from Dr. Randall. We remain hopeful that we can work out an agreement with NMSU to receive the strains. However, we are now also entertaining the possibility that we never might, which frustrates not only our project but also undercuts the need that was expressed and recorded in the notes of the PBTS meeting that was held on May 23 to “perform Koch’s postulates with the original isolates.” It also means that the claim that *Rhodococcus* causes PBTS remains unchallenged.

Uncertain about when we would receive the original isolates from NMSU, we postponed hiring personnel on the project until now. We have requested a no-cost extension of our 2017 funds into 2018 and we will be hiring a postdoc to work and expand on Objective 4, for which we will use protocols now available in the Leveau and Trouillas labs to extract and interrogate by DNA-based means the microbial communities that associate with healthy and PBTS-symptomatic pistachio trees. More specifically, we will be seeking culture-independent, consistent association of *fas* genes and other microbial gene markers with symptomatic tissue from PBTS trees. The discovery of such markers will be guided by the CPRB-funded *Rhodococcus* genome project (PI Trouillas).

CONCLUSION AND APPLICATIONS

Progress on this project was delayed because we did not obtain the biological material that is needed for Objectives 1-3. We are realigning the project in anticipation of not receiving this material. We have asked CPRB for a no-cost extension on the project, which will allow us to develop original Objective 4 into a culture-independent approach to Koch's postulate of consistent association, seeking microbial gene markers that are specific to symptomatic tissues on PBTS trees.