Characterizing novel bacterial isolates that inhibit the growth of gall-forming phytopathogenic bacteria

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Agrobacterium tumefaciens and Rhodococcus fascians are phytopathogenic bacteria

Agrobacterium tumefaciens and Rhodococcus fascians are plant pathogenic bacteria that can induce crown and leafy galls on plants, respectively. Infection by these bacteria results in disfigured, unsellable plants, leading to significant economic losses to the nursery industry. A. tumefaciens and R. fascians are capable of infecting a broad range of plants by co-opting native plant systems to induce gall formation. However, they use different mechanisms. A. tumefaciens, a Gram-negative bacteria, causes disease by transferring its DNA into the plant cell. The expression of this DNA leads to the production of hormone-producing enzymes that disrupt the native levels in the plant, initiating the formation of the tumor-like galls (Figure 1A and 1B). R. fascians, a Gram-positive bacteria, produces its own cytoxins that can disrupt native levels, resulting in leafy galls (Figure 1C and 1D).

Isolate Z30 can reduce symptoms of R. fascians in planta

We tested the ability of isolate Z30 to protect Nicotiana benthamiana plants against R. fascians D188 using a seedling root inhibition assay. R. fascians D188 significantly inhibit roots growth (Figure 4A, 4B). Isolate Z30 does not effect root length when it is inoculated singly onto plants. We tested 5 pre-treatment times (0, 2, 4, 8, and 24 hours). While all treatments had a positive effect against R. fascians D188, pre-treatments of at least 4 hours prior to inoculation were the most effective (Figure 4B).

A novel bacterium discovered in the lab inhibits the growth of both Agrobacterium and Rhodococcus.

We discovered a new bacterium as a contaminant growing on an agar plate that could inhibit the growth of R. fascians (Figure 2A). We tested the ability of this bacterium, Z62, to inhibit the growth of Agrobacterium tumefaciens C58 (Figure 2B) and confirmed that its inhibitory capacity against R. fascians D188 (Figure 2C). We also identified additional, closely related, isolates from a culture collection. These included isolates with varying abilities to inhibit the growth of various Agrobacterium spp. and Rhodococcus spp. in vitro (Table 1), including those lacking any inhibitory capacity (Figure 3).

Characterization of isolate Z62 and its protective effect against A. tumefaciens

We are using two assays to measure the protective ability of isolate Z62 against infection by A. tumefaciens C58.

Using N. benthamiana plants, we are testing whether pre-treatment of stems with isolate Z62 can prevent the formation of crown galls (Figure 5A, 5B).

We are also using a carrot disc assay (Figure 5C) to measure the protective effect of isolate Z62. These experiments are ongoing.

We identified closely related isolates with varying abilities to inhibit R. fascians and A. tumefaciens growth in vitro

We sequenced and assembled the genomes of 20 isolates of bacteria closely related to Z62. Single nucleotide polymorphisms (SNPs) were identified by aligning sequencing reads against Z31 (REF). Figure 3 suggests that the best assembled genome. A maximum likelihood SNP tree was generated (Figure 3A). Interestingly, this tree shows that isolates with inhibitory abilities are closely related to those that cannot inhibit either A. tumefaciens C58 (Figure 3B) or R. fascians D188 (data not shown) in vitro. We are currently using comparative genomics analyses to identify differences between these isolates with the goal of identifying the pathways responsible for the inhibitory mechanism. We are also sequencing the genome of isolate Z62.

Ongoing work, future work, and goals

- Isolate and identify the compound(s) of interest responsible for the inhibitory phenotype of this bacteria.
- Comparative genomics analyses to identify potential secondary metabolite pathways and/or biosynthesis genes.
- Generate mutants in genes of interest to confirm and characterize the mechanisms of growth inhibition and plant protection.
- Explore the efficacy of this bacteria for field, nursery, or greenhouse use as a biocontrol agent against A. tumefaciens and R. fascians.

Acknowledgments

This project is supported by USDA SSCRI Grant #2014-51181-22384. MSB received personal funding from the URISC program at OSU to perform this work. I would like to thank the following people for their contributions to this work: Edward W. Davis II, Dr. Alexandra Weisberg, Skylar Fuller, as well as all the members of the Chang lab for their continued support.

References


Supporting Information

- Figure 1: Symptoms caused by phytopathogenic Rhodococcus fascians and Agrobacterium tumefaciens.
- Figure 2: A novel bacterium was discovered as a contaminant on a plate of transformed R. fascians D188. This bacteria has shown the capacity to inhibit the growth of both phytopathogens in vitro.
- Table 1: Z62 was tested against various isolates of each phytopathogen, with representatives from various clades within their respective taxonomic hierarchies.
- Figure 3: Closely related isolates have different abilities to inhibit phytopathogen growth in vitro. A. Maximum likelihood tree generated from a SNP alignment with Z31 as the reference. Bootstrap values are labeled at the nodes. B. Isolates Z10, Z25, Z30, and Z62 have varying abilities to inhibit the growth of Agrobacterium tumefaciens C58.

Figure 1: Symptoms caused by phytopathogenic Rhodococcus fascians and Agrobacterium tumefaciens. A. Apple root stock with large crown gall caused by A. tumefaciens. B. Rhododendron with crown gall enveloping majority of the stem. C. Nicotiana benthamiana root system infected with R. fascians D188. D. N. benthamiana with a leafy gall caused by R. fascians D188. Arrows indicate leafy gall.