PCR detection of pathogenic *Rhodococcus fascians* and *Agrobacterium tumefaciens* on herbaceous perennials

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**Introduction**

A wide variety of herbaceous perennials are susceptible to infections by *R. fascians* and *A. tumefaciens*, producing symptoms of leafy gall, shoot proliferation or tumors. These infections have had a major economic impact on the nursery industry in the past few years. Virulent and avirulent strains are found in both species, with genes for virulence found on an Ri or Ti plasmid respectively. Determining the causative agent for disease must therefore go beyond identification of the bacteria to include inoculations to indicator plants or detection of genes for virulence. It has often been difficult to isolate *R. fascians* from infected plants with leafy galls and the presence of Agrobacteria on these same plants has often led to confusing results. Misidentification of the infectious agent is possible if Agrobacteria are isolated but not tested for virulence. Only those bacteria that can reproduce the original symptoms can be confirmed as causing the disease. A rapid, sensitive and accurate method would be extremely useful for timely disease diagnosis. PCR has been used in a wide variety of plant diseases to detect pathogenic organisms.

The objective of this study was to develop PCR primers that would detect and discriminate between pathogenic *R. fascians* and *A. tumefaciens* strains.

![Figure 1a. Dicentra with shoot proliferation (infected on left, control on right)](image)

**Materials and Methods**

Primer sets were designed from cytochrome p450 and tas-1 region of the Ri plasmid in *R. fascians* and from the Ti region of the Ti plasmid in *A. tumefaciens*. These primers were used to amplify DNA from known virulent and avirulent strains in our culture collection as well as bacteria isolated from plants with tumors, shoot proliferation or leafy galls. Bacterial strains were grown on Mannitol Glutamate Yeast extract medium (MGY) for two days and a loopful of bacteria suspended in 0.75 ml sterile ultrapure water. DNA was extracted using InstaGene (BioRad, Hercules, CA).

For plants infected with *R. fascians*, 200 mg of tissue were suspended in 3 ml of saline amended with 0.2 mg/ml cycloheximide for 24 to 48h at room temperature. For plants suspended of harboring *A. tumefaciens*, tumors were surface sterilized, then tissue within the tumor was cut into small pieces and placed into 3 ml's of crown gall wash medium (MGY) for two days and a loopful of bacteria suspended in 0.75 ml sterile ultrapure water. DNA was extracted using InstaGene method. DNA templates were stored at -20C. The same plant wash methods were used to detect *R. fascians* from asymptomatic tissue on *Nemedia* and *A. tumefaciens* on *Argyranthemum*.

PCR was effective in amplifying DNA from pathogenic *R. fascians* strains and detecting and discriminating between the two bacterial species in plant washes from a variety of plant hosts. Based on the results of this study, PCR should be an effective method for rapid diagnosis of crown gall or leafy gall in herbaceous perennials. PCR is also effective in detecting *R. fascians* and *A. tumefaciens* on asymptomatic plant material. Many nurseries rely on propagation by cuttings for rapid production of new plants. This could lead to widespread distribution of either bacterium before any symptoms are detected. PCR could be useful in screening stock plants to detect these two pathogens.

**Results**

*R. fascians* was detected in 29 symptomatic plants inoculated with 10 different strains. The bacteria were re-isolated from *Oenothera*, *iberis*, *Tiarella*, *Erysimum*, *Campanula*, and *Veronica*. The DNA extracted from these bacteria was amplified by the p450 primers and produced bands of the same size on gels as the original bacterial DNA.

![Figure 3a. Nemedia with shoot proliferation.](image)

![Figure 3b. Argyranthemum with tumors.](image)

**Discussion**

PCR was effective in amplifying DNA from pathogenic *R. fascians* and *A. tumefaciens* strains and detecting and discriminating between the two bacterial species in plant washes from a variety of plant hosts. Based on the results of this study, PCR should be an effective method for rapid diagnosis of crown gall or leafy gall in herbaceous perennials. PCR is also effective in detecting *R. fascians* and *A. tumefaciens* on asymptomatic plant material. Many nurseries rely on propagation by cuttings for rapid production of new plants. This could lead to widespread distribution of either bacterium before any symptoms are detected. PCR could be useful in screening stock plants to detect these two pathogens.

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