

Determining the Environmental Fate of the Biological Control Organism, *Pseudomonas aureofaciens* through 16SrDNA Gene Probing

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- Objective** The objective was to determine the environmental fate of *P. aureofaciens* in the turfgrass ecosystem through DNA amplification coupled with nucleic acid hybridization.
- Rationale** Biological organisms for the prevention of turfgrass diseases are now available and in widespread use. Biological control is achieved through antagonistic action of one organism toward another such as a pathogen or weed. At present, no genetically engineered organisms are available in the turfgrass industry, however, as advances in biotechnology increase the activity of wild type (natural occurring) organisms through genetic manipulation, engineered varieties will likely enter the market. Although not genetically engineered, the biological control organism *P. aureofaciens* may serve as a predictive model for the behavior of future organisms that may potentially harbor altered genetics and thus, require sensitive post-application monitoring.
- How it was done** Approximately 10^6 cfu ml⁻¹ of *P. aureofaciens* was applied nightly to an established stand of creeping bentgrass through the existing irrigation system. The bacteria were applied in 0.1 inch of water. To determine the environmental fate of the bacteria, samplings of the turfgrass leaves, thatch, and soil (~1 inch depth) were made periodically. DNA was extracted from each matrix and subsequently PCR amplified using primers designed to amplify selected regions of the 16S rDNA of *Pseudomonas* spp., including *P. aureofaciens*. An oligonucleotide probe was developed by aligning the sequence of the *P. aureofaciens* 16S rDNA amplified by the primers mentioned above with the sequences of other closely related Pseudomonads. A variable region within the amplified sequence provided adequate dissimilarity between the target organism and related organisms to allow the design of a 21 base oligonucleotide probe specific to *P. aureofaciens*. The degree of probe specificity was assessed by testing the hybridization of the probe to several closely related Pseudomonads as well as genera of more distant relation. A dilution series of *P. aureofaciens* was inoculated into samples of turfgrass leaves, thatch, and soil followed by DNA extraction and probe hybridization in order to determine the limit of detection for the organism in the turfgrass environment. A qualitative fate assessment of *P. aureofaciens* was determined using dot-blot hybridizations to PCR product derived from leaves, thatch, and soil. In brief, 1 ul of each PCR product was applied to a neutral membrane, fixed with UV irradiation, and probed with the probe labeled with the digoxigenin protein.
- Results to date** Fig. 1 shows the results of adding dilutions of *P. aureofaciens* to leaves, thatch, and soil followed by probe hybridization. The results indicate that at least 10^3 cells per gram dry weight, 10^3 cells, and 10^2 cells are detectable in the leaves, thatch, and soil, respectively. Signal strengths suggest that more dilute samples are assumed to be within the limits of detection.

Testing of the oligonucleotide probe against other *Pseudomonads* indicates sufficient specificity to detect *P. aureofaciens* amongst other closely related bacteria (Fig. 2).

Dot blot hybridizations to leaf surface, thatch, and soil bacterial DNA suggest that the majority of the applied bacteria are collected on the leaf surface with a lesser amount entering the thatch layer (Fig. 3). No hybridization signal was detected in soil-derived PCR products suggesting that most of the *P. aureofaciens* is effectively filtered by the canopy and thatch layer.

From an environmental standpoint, the turfgrass ecosystem effectively arrests the movement of the bacteria after it is applied. This result also proves positive for the use of the organism as a biological control as the intended pathogen, dollar spot (*Sclerotinia homoeocarpa*), is most damaging when active in the turfgrass canopy and upper thatch regions. Thus, the usefulness of *P. aureofaciens* as a biological control organism, assuming the organism is, in fact, active in the turfgrass environment, couples well with its ultimate environmental fate.

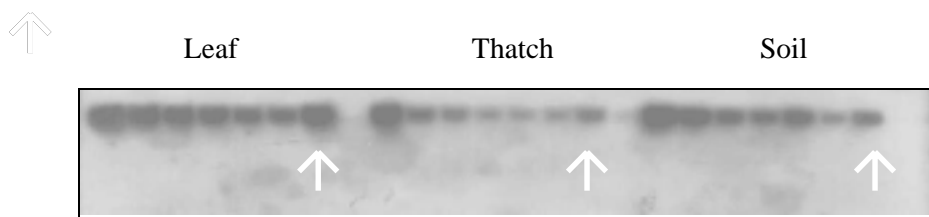


Figure 1. The lowest dilution (white arrows) of *P. aureofaciens* inoculated into turfgrass leaves (10^5 cells per gram dry weight), thatch (10^3 cells), and soil (10^2 cells) is detectable following hybridization with the *P. aureofaciens* probe.

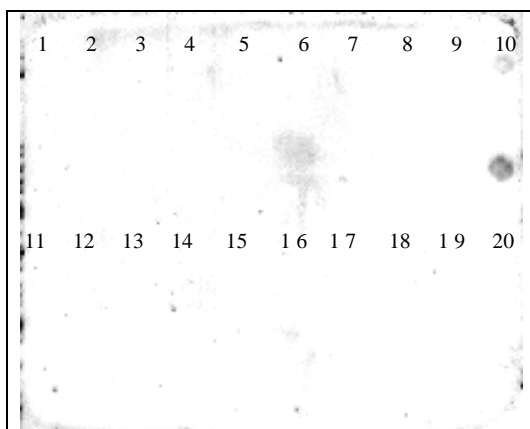


Figure 2. Dot blot hybridization test of *P. aureofaciens* probe specificity. Blot assignments: 1. *P. syringae* pv. tomato, 2. *P. cepacia*, 3. *P. aeruginosa*, 4. *P. putida* AC10R, 5. *P. desmolytica* 1123, 6. *P. fluorescens* 55, 7. *P. stutzeri*, 8. *P. testosteroni* 31, 9. *P. viridiflava* pv5, 10. *P. aureofaciens*, 11. *Comomonas testosteroni*, 12. *Alcoligenes* sp., 13. *Ralstonia* sp., 14. *R. eutrophus*, 15. *P. putida*, 16. blank, 17. *P. desmolytica* 1123, 18. *P. syringae* pv 61, 19. *P. fluorescens* B2265, 20. blank.