Identification of Parasitic Bacteria as Biological Control Agents Against Summer Patch Disease

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Goals:

- *Isolate and identify bacteria which can colonize and parasitize the "mycelia" of Magnaporthe poae, the causal agent of summer patch disease.*
- *Screen isolated bacteria for disease control potential using controlled growth chamber and field studies.*

Cooperator:

Dr. Bruce Clarke

Summer patch disease is caused by the ectotrophic, root-feeding fungus, *Magnaporthe poae*. The disease is extremely damaging to turfgrass, affecting cool-season varieties under conditions of high soil temperature and high water potential. Disease development is enhanced by conditions that contribute to turfgrass root stress, such as low mowing heights or compacted soil. Our primary objective is to investigate the use of beneficial bacteria for control of summer patch and other diseases of turfgrass.

Stenotrophomonas (*Xanthomonas*) *maltophilia* 34S1 (Sm34S1) was previously identified as a biological control agent capable of controlling summer patch disease. Greenhouse/growth chamber studies indicated that Sm34S 1 reduced foliar symptoms on Kentucky bluegrass by as much as 70% compared to untreated disease controls. When Sm34S 1 was applied to plants on a repeated basis, summer patch was suppressed at high, sustained levels.

Colonization studies suggested that Sm34S1 populations should be established within the turfgrass rhizosphere at levels above 10^7 cfu/g sample during a two week period, and should remain above 10^5 cfu/g sample to achieve effective control. Sm34S1 was applied to pathogen-inoculated field plots located in a three year old Kentucky bluegrass stand that received minimal maintenance during the summer of 1995.

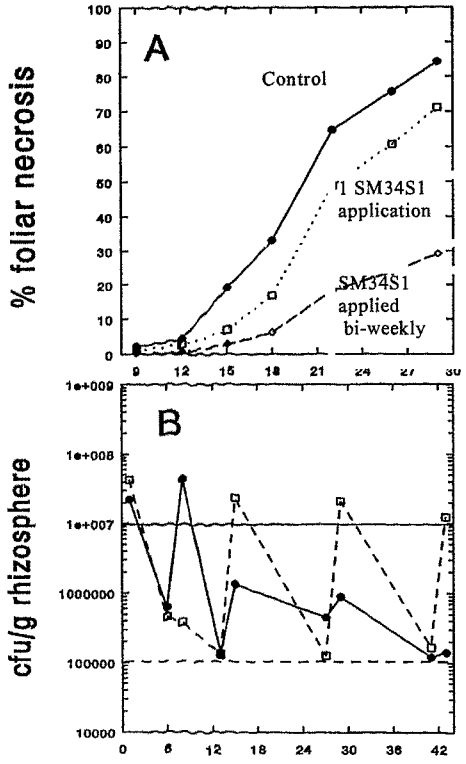

Figure 10. (A) Summer patch suppression on Kentucky bluegrass by repeated applications of SM34S1 (dashed line) in greenhouse/growth chamber studies. (B) Rhizosphere populations of SM34S1 in rootzone: solid line is one application, dashed line is bi-weekly applications.

Summer patch symptom development was not suppressed by Sm34S1during that year. Population studies indicated that Sm34S1 was maintained at levels between 10^4 and 10^7 cfu/g sample. Sm34S1 was applied to pathogen inoculated field plots in 1996 consisting of annual bluegrass/bentgrass green. Summer patch symptoms did not develop in field plots during 1996. Studies indicated that Sm34S1 populations fluctuated in the turfgrass rhizosphere over a range greater than that observed in 1995; however, on occasion, populations were established above the critically determined level of 10^7 cfu/g sample.

A single chitinase gene was cloned from Sm34S1 and the nucleotide sequence was determined. The gene encoded a single polypeptide of cat 1.6 kb, and was associated with a protein of 51.1 kdal in size. Site directed mutagenesis of the gene in 34S1 resulted in loss of chitinase activity, and a significant reduction in biocontrol of summer patch by this organism.

Chitinase activity and biocontrol of summer patch was restored when the cloned gene was reintroduced into the mutant. Studies indicated that chitinase was expressed under conditions of nutrient stress and in the presence of chitin. These studies provide strong evidence for the role of chitinase in biocontrol activity by 34S 1, and information towards understanding the conditions in which the gene is expressed.

Previously isolated biocontrol strains that appeared similar to *S. maltophilia* 34S1 were compared on a taxonomic basis. Fatty acid analysis (MIDI) and nutritional utilization (Biolog) suggested that two isolates, N4-7 and N4-15, previously recovered from the turfgrass rhizosphere and demonstrated to have summer patch suppressive abilities, were closely related *Stenotrophomonas*, *Xanthomonas* and *Xylella* species.

Serological tests using polyclonal antibodies made against N4-7 indicated relatedness to *Xylella* and N4-15, but not to *Stenotrophomonas*. Comparisons of 16s rDNA sequences confirmed the relatedness of both N4-7 and N4-15 to *Xylella* and *Stenotrophomonas*. However, N4-7 appeared most closely related to an unidentified, hydrothermal vent eubacterium.