# Development of Typhula Species Specific PCR Primer

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In the last issue (July/August, 2000) of *The Grass Roots*, we talked about how DNA marker technology can be utilized to understand genetic relationship or diversity (fingerprinting and population structure of fungal pathogen) of any living organisms, such as Typhula fungal species that cause snow mold.

Gray snow mold caused by *T. incarnata* and speckled snow mold caused by *T. ishikariensis* complex are major problems for golf course superintendents in Wisconsin and the Great Lakes Region. In this issue I will introduce research results of the successful development of PCR (polymerase chain reaction) primers, which are based on the sequences of the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (rDNA), distingushing Typhula species (*T. ishikariensis* complex and *T. incarnata*).

The ITS sequences have been widely used to compare various fungal taxa, to differentiate Fusarium sp. and *Rhizoctonia solani* AG-2-3. As a reminder from the last issue, RAPD (random amplified polymorphic DNA) marker was used to understand genetic relationship among Typhula isolates, the three genetically distinct groups (A, B, and C) within *T. ishikariensis* complex that were found in Wisconsin isolates. Our lab's first goal was to design a PCR primer differentiating Typhula species.

We all remember Dr. Steve Millett, who graduated from Plant Pathology, Univ. of Wisconsin-Madison. His PhD research topic was on distribution and characterization of Typhula species using sclerotial morphology, monokaryon x dikaryon mating experiment with known tester isolates, and

| Variable | PCR | RAPD |
|---|---|---|
| Length of primer sequence | 15 - 25 | 8 - 10 |
| Number of amplifying band | one | 10 - 15 |
| Specificity | High | Low |
| Reproducibility across lab | High | Low |
| Primer sequence | Two primers of known nucleotide sequence | Single primer of arbitrary nucleotide sequence |

Figure 1. A gel profile of Typhula fungal isolates using two molecular marker types, PCR and RAPD. Note multiple bands were amplified in RAPD reaction but a single band in PCR reaction.

DNA sequence comparison of ITS regions of rDNA of Typhula species. From the DNA sequences of ITS region performed by Millett (1999), Typhula species-specific PCR primers were designed. Then they were tested to confirm the validity using *T. ishikariensis* and *T. incarnata* isolates collected mostly from Wisconsin and a few from other countries. All of the samples (83 and 48 of *T. ishikariensis* and *T. incarnata*, respectively) tested so far have been positive. Our lab successfully developed PCR primers amplifying a single bright band compared to multiple bands in RAPD reaction (refer in figure 2, Jung, 2000) for each of *T. ishikariensis* and *T. incarnata* species. PCR primers separating two diverse groups A and C (Biological Species Group I) from group B (Biological Species Group II) described in figure 3 (Jung, 2000) were also designed.

Rapid and accurate diagnosis of Typhula species pathogenic to turfgrasses is an important part of managing the diseases that they cause. Furthermore, it is not easy to identify the *T. ishikariensis* complex isolates due to taxonomy confusion among researchers and maybe complexity of Typhula organism itself. For these reasons our results are valuable not only for golf course superintendents but for researchers as well.

What are differences between PCR and RAPD marker? Some variables are compared in Figure 1.

RAPD gel picture

PCR gel picture

The next step is to plan an experiment that answers the question, "Is there any difference in fungicide sensitivity among each genetically diverse groups of *T. ishikariensis* complex?" Then those isolates will be used for the inoculation of turfgrass species in the field condition in order to associate with in vitro results.

What we are learning from present and future results is to aid golf course superintendents in reducing their fungicide input by providing information of proper fungicides controlling Typhula isolates.

**References**

Millett, S. 1999. Distribution, biological and molecular characterization, and aggressiveness of Typhula snow molds of Wisconsin golf courses. Ph.D. Dissertation, Univ. Wisconsin, Madison, WI.

Jung, G. 2000. Application of DNA marker technology to turfgrass pathology research. *The Grass Roots* Volume XXIX (4):37-41.