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Investigations of dothistromin gene expression
in
Dothistroma septosporum
and
the putative role of dothistromin toxin.

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Abstract

Dothistroma septosporum causes pine needle blight, a foliar disease currently causing epidemics in the Northern hemisphere. *D. septosporum* synthesizes dothistromin, a mycotoxin similar in structure to the aflatoxin (AF) precursor versicolorin B. Orthologs of AF genes, required for the biosynthesis of dothistromin, have been identified along with others that are speculated to be involved in the same pathway. The dothistromin genes are located on a mini-chromosome in *Dothistroma septosporum* but, in contrast to AF genes, not in a continuous cluster.

The aim of this study was to increase knowledge of the biological role of dothistromin, which was previously a suspected pathogenicity factor. To identify putative roles of dothistromin, the dothistromin gene expression was investigated and green fluorescence protein (GFP) reporter gene strains of *D. septosporum* were developed.

Expression analyses of dothistromin genes revealed co-regulation. More surprisingly, dothistromin is produced at an early stage of growth and gene expression is highest during exponential growth. This is fundamentally different to the late exponential/stationary phase expression usually seen with secondary metabolites such as AF. Strains with a dothistromin gene (*dotA*) promoter-regulated GFP confirmed early expression of the toxin genes, even in spores and germ tubes. Parallel studies with transformants containing a GFP-DotA fusion protein suggest spatial organization of dothistromin biosynthesis in intracellular vesicles. The early expression of dothistromin genes led to the hypotheses that dothistromin is either required in the early stage of the plant/fungi interaction, or for inhibiting the growth of competing fungi.

Constitutive GFP strains helped to determine that dothistromin is not a pathogenicity factor. However, a putative role of dothistromin in competition with other fungi, including pine-colonizing species, was detected, supporting the second hypothesis. It was shown that dothistromin-producing strains appear to have a competitive advantage which is lacking in dothistromin-deficient strains. However, some competitors were not affected and have potential as biocontrol agents.

In summary, this work has led to the discovery of an unusual pattern of regulation of a secondary metabolite, has made substantial progress in identifying the biological role of dothistromin, and has indicated potential for biocontrol of *Dothistroma* needle blight.

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