Regulation of dothistromin toxin biosynthesis by the pine needle pathogen *Dothistroma septosporum*.

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Abstract

Dothistromin is a virulence factor produced by the fungal pine needle pathogen *Dothistroma septosporum*. It is similar in structure to a precursor of aflatoxin and sterigmatocystin. Unlike most secondary metabolite genes in fungi, the genes for dothistromin biosynthesis are not clustered but spread over six loci on one chromosome. Another characteristic feature of dothistromin synthesis is that dothistromin is produced mainly during the early exponential growth phase in culture. These unusual features have been proposed to be adaptations for the biological role of dothistromin in the disease process. It was therefore of interest to determine whether the regulation of dothistromin production in *D. septosporum* differs from the regulation of aflatoxin and sterigmatocystin in *Aspergillus* spp. and to address the question of whether genes in a fragmented cluster can be co-regulated.

The availability of the *D. septosporum* genome facilitated identification of orthologs of the aflatoxin pathway regulatory genes *aflR*, *aflJ* and the global regulatory genes *veA* and *laeA*. These genes were functionally characterised by knockout and complementation assays and the effects of these mutations on the expression of dothistromin genes and the production of dothistromin were assessed.

Inactivation of the *DsAflR* gene (*ΔDsAflR*) resulted in a 10^4 fold reduction in dothistromin production, but some dothistromin was still made. This contrasted with *ΔAflR* mutants in *Aspergillus* species that produced no aflatoxin. Expression patterns in *ΔDsAflR* mutants helped to predict the complete set of genes involved in dothistromin biosynthesis.

AflJ was proposed to act as a transcriptional co-activator of AflR in *Aspergillus* spp. Disruption of *DsAflJ* resulted in a significant decrease in dothistromin production and dothistromin gene expression. Interestingly the expression of *DsAflR* was not

affected by deleting *DsAflJ,* while conversely *DsAflJ* transcript levels increased significantly in a *DsAflR* mutant compared to the wild type. Heterologous complementation with *A. parasiticus, A. nidulans* and *C. fulvum AflJ* failed to revert the dothistromin level to wild type suggesting species-specific function of AflJ.

VeA is an important regulator of secondary metabolism and development in fungi. Inactivation of the *D. septosporum* ortholog (*DsVeA*) resulted in reduced dothistromin production and showed the influence of DsVeA on the expression of other secondary metabolite backbone genes. Asexual sporulation was reduced but mutants were not compromised in pathogenicity. Overall, *D. septosporum* DsVeA showed functional conservation of the usual role in fungi.

LaeA is a global regulator of secondary metabolism and morphogenetic development, first identified in *Aspergillus nidulans*. Unexpectedly, DsLaeA exhibited an unusual repressive function on the dothistromin pathway and *DsLaeA* mutants exhibited an extended period of dothistromin production compare to WT *in vitro.* The mutation of *DsLaeA* showed varied responses in expression of other secondary metabolite genes and had differences in sporulation and hydrophobicity compared to the wild type.

Results from this study suggest that that some aspects of secondary metabolite gene regulation, such as coordinated control by the pathway specific regulator DsAflR, are conserved in the fragmented dothistromin gene cluster. However, DsAflJ appears to have a species specific role. The global regulators DsLaeA and VeA had conserved roles but most intriguing was that DsLaeA acted as a repressor of dothistromin biosynthesis, deviating from its usual function in other ascomycetes.

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The Author

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