Overexpression of Secondary Metabolism Genes from *Magnaporthe grisea* and *Beauveria bassiana*

Specialty: Fungal Biotechnology

Abstract

Magnaporthe grisea is the most devastating fungal pathogen of rice. Certain rice strains are resistant to strains of *M. grisea* that produce a hybrid polyketide synthase – non-ribosomal peptide synthetase (PKS-NRPS) called avirulence conferring enzyme1 (ACE1) in appressoria. Heterologous expression of the *ace1* gene was attempted in *Aspergillus oryzae* in order to discover the avirulence signal. Initial failure to produce ACE1 was investigated by expressing truncated versions of *ace1* fused at their 3'-termini with the *egfp* reporter gene. This identified mis-splicing of intron-2 as the cause, and removal of this intron led to successful heterologous expression of ACE1 in *A. oryzae* and discovery of its polyketide product, 12,13-dihydroxymagnaporthepyrone. A similar product, 10,11-dihydroxymagnaporthepyrone was identified in the mycelium of *M. grisea* clones that had been transformed to overexpress *ace1* constitutively. These polyketide-only compounds suggest that the NRPS module of ACE1 might be redundant or require a special amino acid biosynthesised only in appressoria.

Beauveria bassiana strains produce tenellin and desmethylbassianin (DMB), hybrid polyketide-tyrosine compounds synthesised by tenellin synthetase (TENS) or its homologue DMB synthetase (DMBS) together with nearly identical post-PKS modifying enzymes. Hexaketide DMB and pentaketide tenellin differ because DMB synthesis involves one extra cycle of chain extension and one less *C*-methylation. Heterologous co-expression of TENS with the enoyl reductases (ER) from the tenellin or DMB clusters, produced pretenellin A, proving that *trans*-acting ER do not control programming of highly reducing (HR) fungal PKS. Progressive substitution of TENS functional domains with their DMBS counterparts and heterologous co-expression with either ER resulted in production of compounds with changes that could be mapped to specific domains. Individual domain swaps showed that the *C*-methyltransferase domain controls its own programming, while the ketoreductase (KR) domain was found to be responsible for the chain-length variation between tenellin and

DMB. Heterologous co-expression of TENS with the KR domain swapped from DMBS together with all the other tenellin biosynthetic enzymes produced bassianin, an "extinct metabolite" previously described in *B. bassiana* but no longer detectable in numerous isolates.

This research highlighted that correct intron splicing cannot be relied upon to occur during heterologous expression of every fungal gene in other fungal hosts. Recognition of this problem with respect to the *ace1* gene allowed the discovery of the ACE1 polyketide product. This is a significant step towards identifying the ACE1 signal, with potential biotechnological importance in combating *M. grisea* infection of rice. Finally, heterologous expression of TENS and DMBS domain-swapped chimaeric enzymes led, for the first time, to the discovery of crucial biosynthetic programming determinants in HR PKS-NRPS systems.