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Regulation of Paxilline Biosynthesis in Penicillium paxilli

A Thesis presented in partial fulfilment of
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Emily Jane Telfer

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For Ally & Ian

My best friends and my inspiration.

Abstract

Production of the indole-diterpenoid paxilline was examined in the filamentous ascomycete *Penicillium paxilli.* Paxilline is a secondary metabolite, that is synthesised via a specific secondary metabolite biosynthetic pathway. The primary precursors of paxilline biosynthesis, mevalonate and isopentenyl pyrophosphate, are synthesised via the isoprenoid pathway and the paxilline biosynthetic pathway branches from isoprenoid biosynthesis after the synthesis of farnesyl pyrophosphate. The enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co) reductase is the rate limiting step of the isoprenoid biosynthesis. Genes for (*hmg*) and β-tubulin (*tub-2*), were isolated from a genomic DNA libray and characterised by DNA sequencing and RT-PCR. The steady state mRNA levels of *hmg* and *tub-2* were compared with genes isolated from the paxilline biosynthetic gene cluster, using a semi-quantitative RT-PCR gene expression assay. A distinct pattern of expression was identified for genes involved in the biosynthesis of paxilline. Increased expression of these genes occurs 36 h prior to the detection of paxilline in liquid culture.

P. paxilli physiology and paxilline production was analysed in liquid culture after the development of reproducible growth conditions that results in the formation of homogeneous loose hyphal fragments and detectable paxilline after 72 h. The morphology of *P. paxilli* in paxilline-inducing media was examined microscopically and key physiological markers, culture pH and biomass accumulation, were also analysed. Paxilline levels in both mycelia and culture supernatant were analysed with HPLC and TLC. This confirmed that paxilline is not released into the media until 144 h when large scale autolysis is observed. Initial experiments to examine paxilline production in cultures supplemented with a biological buffer suggest that phase switching between primary growth and secondary growth may be triggered by changes in ambient pH. The presence of alternative carbon sources also affected the rate of paxilline production and preliminary results indicate that biosynthesis of paxilline may be under carbon catabolite repression by glucose.

Levels of HMG CoA reductase are known to be regulated at many levels, including mRNA transcription, protein inactivation and protein degradation, in response to excess sterols. A number of putative sterol response elements (SRE), which control transcription of *hmg* in higher eukaryotes, where identified in the 5' UTR of *hmg* from *P. paxilli*. In higher eukaryotes, the extremely complex 5' UTR of *hmg* has been proposed as the site of regulation for biosynthesis of non-sterol end-products. This complexity appears to be conserved in the 5' UTR of *hmg* from *P. paxilli* and another filamentous fungus *Neotyphodium lolii* Lp19. Intronic sequences are spliced from the 5' UTR of both genes and there are additional intronic sequences present that could produce alternative transcripts. At least two different *hmg* transcripts were identified from *P. paxilli* with 5' RACE. The mechanism by which these alternative transcripts arise is unclear at present, but could involve alternative splicing of the 5' UTR intron or initiation of transcription from alternative start sites.

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