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# Functional analysis of *Penicillium paxilli* genes required for biosynthesis of paxilline

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### ABSTRACT

Paxilline belongs to a large, structurally and functionally diverse group of indole-diterpenes and is synthesised by the filamentous fungus *Penicillium paxilli*. A gene cluster for paxilline biosynthesis in *P. paxilli* has been identified and characterised. However, none of the steps proposed in the biosynthesis of paxilline or paxilline-like indole-diterpenes have been validated. In some diterpene-producing filamentous fungi, including *P. paxilli*, two distinct copies of geranylgeranyl diphosphate (GGPP) synthase, that catalyses the committed step in diterpene biosynthesis, have been identified. However, the biological significance of the presence of two distinct GGPP synthases is not known. In this study, biochemical analysis of the paxilline gene products in *P. paxilli* and subcellular localisation of the two *P. paxilli* GGPP synthases, Ggs1 and PaxG, were carried out.

Transfer of constructs containing different combinations of pax genes into a pax cluster negative deletion derivative of P. paxilli identified four Pax proteins that are required for the biosynthesis of a paxilline intermediate, paspaline. These proteins are PaxG, a GGPP synthase, PaxM, a FAD-dependent monooxygenase, PaxB, a putative membrane protein, and PaxC, a prenyltransferase. Using precursor feeding experiments, it was confirmed that the indole-diterpenes paspaline and  $\beta$ -PC-M6 are substrates for the cytochrome P450 monooxygenase, PaxP, and are converted to 13desoxypaxilline. Further, it was confirmed that the indole-diterpene 13desoxypaxilline is a substrate for PaxQ, a cytochrome P450 monooxygenase, and is converted to paxilline. Unlike PaxQ, PaxP is specific for indole-diterpene substrates that have a  $\beta$ -stereochemistry. The detection of the indole-diterpene products was related to the expression of the transgene in the pax cluster negative background.

Reporter fusion studies of the two *P. paxilli* GGPP synthases, Ggs1 and PaxG, showed that the Ggs1-EGFP fusion protein was localised to punctuate structures whose identity could not be established, and the EGFP-GRV fusion

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protein, containing the C-terminal tripeptide GRV of PaxG, was localised to peroxisomes.

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