Functional analysis of PaxP and PaxQ, two cytochrome P450 monooxygenases required for paxilline biosynthesis in *Penicillium paxilli*

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Abstract

The indole-diterpene paxilline is a potent mammalian tremorgenic mycotoxin and a known inhibitor of maxi-K ion channels. The gene cluster encoding the enzymes for the synthesis of this compound was recently cloned from *Penicillium paxilli* (Young *et al.* 2001). The cluster comprises a set of core genes required for indole-diterpene biosynthesis, including two cytochrome P450 monooxygenases, *paxP* and *paxQ*. Targeted deletion of *paxP* and *paxQ* resulted in mutant strains that accumulate paspaline and 13-desoxypaxilline, respectively, confirming that both genes are involved in paxilline biosynthesis. The aim of the current work is to establish *in vitro* that PaxP and PaxQ catalyse the monooxygenation of paspaline and 13-desoxypaxilline, respectively. To achieve this, cDNA copies of both genes were cloned into pGEX-6P-3, to generate pRL2 and pRL4, and the corresponding glutathione-S-transferase (GST) fusion proteins over-expressed in *E. coli*. However, both GST-fusion proteins accumulated as insoluble inclusion bodies when cultures were incubated at 18°C, 25°C and 37°C. Attempts to express a soluble form of the GST-PaxP by co-expressing this fusion with the chaperones, GroES and GroEL, or by expressing in *E. coli*, Origami B, a strain (*trxB*, *gor*, *lacY*) designed to facilitate expression of active and soluble proteins, were unsuccessful. GST-PaxP was able to be solubilised by the addition of 0.25% *N*-laurylsarcosine, and retained some glutathione binding activity, however, the yield was too low to carry out further experiments. GST and thioredoxin fusion expression constructs were designed in which the putative N-terminal trans-membrane region of PaxP and PaxQ was removed to aid solubility in *E. coli*. These N-terminal modified fusion proteins were still expressed as insoluble protein.

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Abbreviations

APS	ammonium persulphate
bp	base pairs
cDNA	complementary deoxyribonucleic acid
CPR	cytochrome P450 reductase
DEPC	diethylpyrocarbonate
DMAPP	dimethyallyldiphosphate
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dsDNA	double stranded deoxyribonucleic acid
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
ER	endoplasmic reticulum
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
FPP	farnesyldiphosphate
gDNA	genomic deoxyribonucleic acid
GGPP	geranylgeranyldiphosphate
GST	glutathione-s-transferase
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
IPP	isopentenyldiphosphate
IPTG	isopropylthiogalactoside
kb	kilobase pairs
LB media	Luria Bertaini media
MCS	multiple cloning site
mRNA	messenger ribonucleic acid
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
PAGE	polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
psi	pounds per square inch
RNA	ribonucleic acid

RT-PCR	reverse-transcriptase polymerase chain reaction
SDS	sodium dodecyl sulphate
SRS	substrate recognition site
TEMED	tetramethylethylenediamine
tRNA	transfer ribonucleic acid
UV	ultra violet
X-Gal	5-Bromo-4-chloro-3-indolyl-beta-D-galactoside

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