

P-024: CLONING THE DITERPENE GENE CLUSTER FOR THE FUNGAL ANTIBIOTIC PLEUROMUTILIN

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The diterpene antibiotic pleuromutilin is produced by a variety of fungi and although it is not used commercially as the natural product, semisynthetic derivatives have been used to treat swine dysentery. The latest pleuromutilin derivative, retapamulin, is effective in humans for treating skin infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. Because of its complex tricyclic structure and important bioactivity, we have focused on identifying the pleuromutilin biosynthetic genes. Our approach relies on recent studies of fungal isoprenoid biosynthesis that suggest that diterpene biosynthetic genes are clustered. Two alternative routes to locating the cluster in the fungus *Pleurotus passeckerianus* have been utilized – a direct approach pursuing the diterpene synthase (DS) gene and an indirect approach via a geranylgeranyl diphosphate synthase (*ggs*) gene. Degenerate primers targeting the DS gene were designed and used with cDNA as template, but no specific PCR products were obtained. Degenerate primers for the *ggs* gene were next used with genomic DNA as template and three distinct PCR products were obtained. A fosmid genomic library has recently been prepared and probes corresponding to the three PCR products have been used to probe the library. Several positive fosmids have been obtained and results of sequencing of the fosmids will be presented.