

Genomics-driven PCR Screening and Isolation of a Cryptic Polyene Gene Cluster in *Pseudonocardia autotrophica*

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The polyene antibiotics, a category which includes nystatin, pimarinin, amphotericin and candicidin, comprise a family of most promising antifungal polyketide compounds, and are typically produced by soil actinomycetes species. The biosynthetic gene clusters for these polyenes have been previously investigated, revealing the presence of highly homologous cytochrome P450 hydroxylase (CYP) genes. Using polyene CYP-specific PCR screening with several actinomycetes genomic DNAs, the *Pseudonocardia autotrophica* strain was determined to contain a unique polyene-specific CYP gene. Genomic DNA library screening using the polyene-specific CYP gene probe revealed a positive cosmid clone which contained a DNA fragment of approximately 34.5 kb. The complete sequencing of this DNA fragment revealed a total of seven complete and two incomplete open reading frames (ORFs), which were found to be highly homologous, but also unique to the previously-known polyene biosynthetic genes. These results suggest that the polyene-specific screening approach may constitute an efficient method for the isolation of potentially-valuable cryptic polyene biosynthetic gene clusters from various rare actinomycetes species