CLONING AND CONFIRMATION OF LAIDLOMYCIN BIOSYNTHETIC GENE CLUSTER IN STREPTOMYCES SP. KCTC 10631BP

Jae Yoon Hwang, Nguyen Phan Kieu Hanh, Doo Hyun Nam*; College of Pharmacy, Yeungnam University, Gyongsan 712-749 Korea; indianblue85@hanmail.net

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Introduction. Laidlomycin is one of the important polyether antibiotics produced by *Streptomyces* sp. KCTC 10631BP isolated from soil samples from Jeonnam, Korea. It shows antimicrobial activity against Gram(+) bacteria including MRSA and VRE. To obtain laidlomycin biosynthesis gene cluster, the library was constructed into SuperCos1 cosmid and screened with specific probes. Three cosmid clones were selected and sequenced. The sequences reveal that the gene cluster contains polyketide synthase(PKS) genes and other functional genes for biosynthesis of laidlomycin. For the confirmation of involvement this gene cluster in laidlomycin biosynthesis, disruption experiments of two genes were performed.
In this work, the organization and gene characters of laidlomycin gene cluster is revealed.

Methods. The construction of cosmid library was performed with SuperCos1 vector into XL1-blue MRF' following protocol of the manufacturer. For screening of three cosmids, Southern hybridization was performed with ^{32}P-labeled probes. [1] DNA sequencing was done by Genotech Co., INC and analyzed by using FramePlot version2.3.2 [5] and NCBI-BLAST program. The gene disruption experiment was performed following the protocol of John Innes Centre. [3]

Results. From the screening, three cosmids were selected. First, using the epoxidase gene fragment as a probe, pSLD-36 cosmid clone was isolated, which contains some essential genes including type I PKS, epoxidase and epoxide hydrolase genes for the biosynthesis of laidlomycin. Next enoyl reductase and epoxide hydrolase I probes were employed for chromosome walking, and new cosmid clone pSLD-BI5 was screened which encodes the downstream region of PKS genes. Finally, pSLD-CII6 clone was isolated using the amplified epoxide hydrolase II gene fragment as a probe. In this cosmid clone, the three PKS genes in upstream region, genes for cyclase, resistance protein, regulator protein and thioesterase were identified. The full length of the laidlomycin biosynthetic gene cluster was larger than 100kb. The gene disruption of LADAVI among the PKS genes and epoxidase gene were done with 1.4kb apramycin disruption cassette and antibiotic assay of extract from wild type and two mutants was tested on *Micrococcus luteus.*

Conclusions. pSLD-36 contained type I polyketide synthase (PKS ;LadAIV, LadAV, LadAVI), epoxidase (LadEI), isomerase I, II (LadIII, LadII) involved in the biosynthesis of laidlomycin. pSLD-BI5 cosmid clone was encoding the downstream region of PKS gene (LadAVIII, LadAVII), and other functional genes like hydroxylase (LadH), acyltransferase (LadAX), aldehyde dehyrogenase (LadDH), and membrane proteins. pSLD-CII6 contained upstream part of PKS (LadAI, LadAII, LadIII), thioesterase (LadAIX), regulator protein (LadRII), resistance protein (LadT), cyclase (LadEII). Around 100 Kb gene was revealed to be highly homologous with the biosynthetic gene cluster for a typical type I PKS polyether antibiotic, monensin, produced from *S. cinnamonensis.* From the antimicrobial activity assay of wild type and mutants, it shown the 100kb gene cluster involved in the biosynthesis laidlomycin

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