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Cloning and Characterization of a Gene Cluster Involved in the Biosynthesis of Polyketide Antibiotic Dihydrochalcomycin in *Streptomyces* sp. KCTC 0041BP

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Dihydrochalcomycin (GERI-155) is produced by *Streptomyces* sp. KCTC 0041 BP isolated from Korean soil. Dihdrochalcomycin is a 16-membered macrolide antibiotic consisting of two deoxysugar moieties at C-5 and C-20 positions of a branched lactone ring. In this study, we report the cloning and characterization of biosynthetic genes involved in dihydrochalcomycin production in *S.* sp. KCTC 0041 BP. The cloning and sequencing of a gene cluster for dihydrochalcomycin biosynthesis revealed a 75.4kb nucleotide fragment containing 31 open reading frames (ORFs). The 63kb region in this fragment was found to contain 25 ORFs. The products of all of these ORFs play a role in dihydrochalcomycin biosynthesis and self-resistance against the compounds synthesized. At the core of this cluster lies a 39.6 kb polyketide synthase (PKS) region was also found. This core region encodes eight modules in five giant multifunctional protein-coding genes (*gerSI-SV*). Each module adds two carbon units to polyketides chain at each cycle of elongation. The loading module and seven extender modules thus found are responsible for the synthesis of 16-macrolide aglycone of dihydrochalcomycin. The genes responsible for the biosynthesis of deoxysugar moieties, D-chalcose and D-mycinose were also found on either side of the PKS genes. The genes for the modification of deoxysugars, their attachement and inactivation inside the cell were also identified. Some of the PKS domains, however, could not be found in this cluster. These were enoylreductase (ER) domain in module 3, and dehydratase (DH) and ketoreductase (KR) domains in module 7 of the cloned PKS. It is believed that these domains are located away from the PKS gene cluster as discdrete enzymes and perform their catalytic function *in-trans*. The discrete catalytic activity of such domains has already been reported in other polyketides producing strains. To confirm the involvement of this gene cluster in dihydrochalcomycin biosynthesis disruption of dehydratase (DH) domain in module 3 of the PKS gene was carried out. The disruption of gene was characterized by southern hybridization. The loss of dihydrochalcomycn production was confirmed by LC-MS (ESI) analysis and antibacterial susceptibility tests. The disruption of targeted gene and resultant loss of dihydrochalcomycin production in the disruptant indicated that the cloned gene cluster is responsible for dihydrochalcomycin production.