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Cloning and Characterization of a Gene Cluster for the Production of Polyketide Macrolide Dihydrochalcomycin in *Streptomyces* sp. KCTC 0041BP

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Abstract Dihydrochalcomycin (GERI-155), produced by *Streptomyces* sp. KCTC-0041BP isolated from Korean soil, is a 16-membered macrolide antibiotic consisting of two deoxysugar moieties at C-5 and C-20 positions of a branched lactone ring. The cloning and sequencing of a gene cluster for dihydrochalcomycin biosynthesis revealed a 63-kb nucleotide region containing 25 open reading frames (ORFs). The products of all of these 25 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 encoding eight modules in five giant multifunctional protein-coding genes (*gerSI-SV*). The genes responsible for the biosynthesis of deoxysugar moieties, D-chalcose and D-mycinose, and their modification and attachment were found on either side of this PKS region. The involvement of this gene cluster in dihydrochalcomycin biosynthesis was confirmed by disruption of the dehydratase (DH) domain in module 3 of the PKS gene and by metabolite analysis.

Key words: Macrolide antibiotic, dihydrochalcomycin, biosynthetic gene cluster, polyketide synthase, *Streptomyces*

Polyketides are a large and highly diverse class of secondary metabolites that include antibacterial, antifungal, anticancer, antiparasitic, antiangiogenesis, and immunosuppressant activities, among others [20, 25]. They are synthesized by a common pathway, in which acetyl, propionyl, or butyryl-CoA monomers are condensed onto a growing chain by a polyketide synthase (PKS) in a process resembling fatty acid biosynthesis [11, 15]. However, the β-keto function at each building unit undergoes varying degree of reduction that confers wide structural diversity to this class of compounds [6, 16].

Dihydrochalcomycin (Fig. 2) is a 16-membered polyketide macrolide antibiotic produced by *Streptomyces* sp. KCTC-0041BP along with two other macrolides, chalcomycin and aldgamycin E, having quite similar aglycone structure [19]. Dihydrochalcomycin has been shown to exhibit balanced antimicrobial activity against Gram-positive as well as Gram-negative bacteria. Similarly to many other macrolide antibiotics, dihydrochalcomycin has been suggested to inhibit protein synthesis by binding to 23S rRNA in the 50S subunit of bacterial ribosome [7]. In this study, the organization of the gene cluster responsible for dihydrochalcomycin biosynthesis is described, based on its 63-kb nucleotide sequence.

Materials and Methods

Bacterial Strains and Culture Conditions

Streptomyces sp. KCTC-0041BP (formerly reported as GERI-155) was used as a source of DNA for the construction of the genomic library. It was routinely grown on ISP2 agar at 28°C. For total DNA isolation, the strain was cultured in YEME medium supplemented with 20% sucrose and 0.5% glycine at 28°C and 200 rpm for 36 h [17]. For conjugal transfer, *S.* sp. KCTC-0041BP was grown in tryptic soy broth, and exoconjugants were regenerated on mannitol-soya (MS) agar supplemented with 25 μg/ml nalidixic acid, and either neomycin (20 μg/ml) and apramycin (50 mg/ml) or neomycin alone [17, 37]. *Escherichia coli* XL-1 Blue MRF' (Stratagene, CA, U.S.A.) was used as a host for cosmid recombinant derivatives and for plasmid subcloning. *E. coli* ET 12567/pUZ8002, a non-methylating (*dam*$^-$ *dcm*$^-$ *hsdS*$^-$) strain, was used as a donor host for conjugal transfer into *S.* sp. KCTC-0041BP [24]. *E. coli* strains were grown

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on Luria-Bertani (LB) medium supplemented with appropriate antibiotics [17, 28].

Vectors and DNA Manipulation

pGEM-T Easy and pGEM-3Zf(+) (Promega, WI, U.S.A.) were the routine cloning vectors for DNA manipulation, and pOJ446 vector was used for the construction of the genomic library [17]. Plasmid, cosmid, and genomic DNA preparation, restriction digestion, DNA fragment isolation, and cloning were performed by following standard procedures [17, 28].

Construction and Screening of Cosmid Library

For cosmid library construction, 5 μg of *S.* sp. KCTC-0041BP chromosomal DNA was partially digested with Sau3AI and ligated into pOJ446 [17], which was pretreated with HpaI, dephosphorylated, and restricted with BamHI. The ligated mixture was packaged with Gigapack® III Gold packaging extract (Stratagene, CA, U.S.A.) and transduced into *E. coli* XL-1 Blue MRF' host. Based on high sequence similarity among ketosynthase domains (KS) of various type I PKS's [13], a pair of degenerate primers, KS-1F (5'-GCCCGGCCGTCACCRTNGAYACNGC-3') and KS-1R (5'-CGCATGGCCATGACCATYTTDATNCAN CC-3'), was designed to amplify the KS fragment. Similarly, primers DW11 (5'-CACTTCGGGGGCGAGTCGC ACG-3') and DW32: (5'-GGGCCGTAGTTGTTGAGC-3') were designed to amplify a part of the dTDP-4,6-dehydratase (4,6-DH) gene involved in the biosynthesis of many deoxysugars [9]. Both amplified KS and 4,6-DH fragments were employed as probes to screen the cosmid library of *S.* sp. KCTC-0041BP for the isolation of the dihydrochalcomycin biosynthesis gene cluster. Hybridization was carried out with each probe at 60°C for 5 h in 20 ml of 2× SSC [28].

DNA Sequencing and Analysis

Full sequencing of cosmid clones and contig assembly were entrusted to Genotech Co. Ltd. (Daejon, Korea). Sequences were analyzed using the FramePlot version 2.3.2 [12]. DNA and deduced protein sequence homology searches of databases were performed with the BLAST program [1]. Multiple alignment was performed using the ClustalW program [32].

Gene Disruption and Confirmation of Loss of Antibiotic Production

To confirm the involvement of the cloned PKS gene in dihydrochalcomycin biosynthesis, disruption of the dehydratase domain in module 3 (DH-3) was attempted by a homologous recombination approach using pKC-1139 plasmid [4]. Thus a 1.9-kb DNA region encoding the targeted DH-3 was amplified from the pMT-49 cosmid using two primers, DH 11-3F (5'-GCAAGCTTCCGGCCGGTGGCGCCATGG-3') containing the HindIII site and DH11-3R (5'-CCGAATTC-CACCAGGACGTGGTCGGGC-3') containing the EcoRI site. A 0.95 kb SalI fragment containing the *aphII* neomycin-resistance gene (*neo*^{r}) from the pFDNEO-S plasmid [10] was introduced into an internal SmaI site of the amplified DH-3 DNA fragment by blunt ligation. The whole construct was ligated into the HindIII and EcoRI sites of pKC-1139 to give pKC-DH, the disruption cassette for insertional inactivation of the targeted DH-3 region. The pKC-DH was finally electrotransformed into *E. coli* ET 12567/pUZ8002, and conjugal transfer to *S.* sp KCTC-0041BP was carried out with slight modification of the established method [17]. After several generations of culture, first at 28°C, then at 37°C, and back again at 28°C, in R2YE medium containing neomycin (20 μg/ml) or apramycin (50 μg/ml) with nalidixic acid, two exoconjugants resistant to neomycin but sensitive to apramycin were screened. To confirm the integration of the *aphII* gene (*neo*^{r}) at the DH-3 region, chromosomal DNA of an exoconjugant (DH6-1) was digested with BamHI and hybridized with the labeled DH-3 and *neo*^{r} gene probes. For metabolite analysis, the supernatants from the 5-d culture of DH6-1 exoconjugant and wild-type (WT) strains in the production medium were extracted with 2× volume of ethyl acetate, dried, and dissolved in methanol [19]. The methanol extracts were finally subjected to LC-MS (ESI) (Thermo Finnigan, CA, U.S.A.) and compared.

RESULTS AND DISCUSSION

Cloning of the Dihydrochalcomycin Biosynthesis Gene Cluster in *S.* sp. KCTC-0041BP

Being a 16-membered macrolide, dihydrochalcomycin was believed to be formed by a type I PKS system. Therefore, to isolate the type I PKS gene cluster along with anticipated deoxysugar biosynthetic genes, the cosmid library was cross-hybridized with [α-^{32}P]-labeled KS and 4,6-DH probes under high stringent conditions. Colony hybridization, restriction analyses, and Southern hybridization of the two probes with the BamHI-digested cosmid DNA of positive clones led to the identification of two overlapping clones, pDB51 and pMT49. Complete sequence analysis of the pDB51 and pMT49 cosmids revealed a 63-kb segment consisting of 25 ORFs involved in the biosynthesis of dihydrochalcomycin and its resistance for the producer organism. Results of these analyses are summarized in Fig. 1 and Table 1. The nucleotide sequence of the whole 75.5-kb region encoding the genes for dihydrochalcomycin biosynthesis has been deposited in the GenBank under the accession number AY118081.

Organization of the Dihydrochalcomycin Biosynthesis Genes

Of the 31 ORFs found in the 75.5-kb nucleotide region, 23 were found to be involved in biosynthesis of dihydrochalcomycin

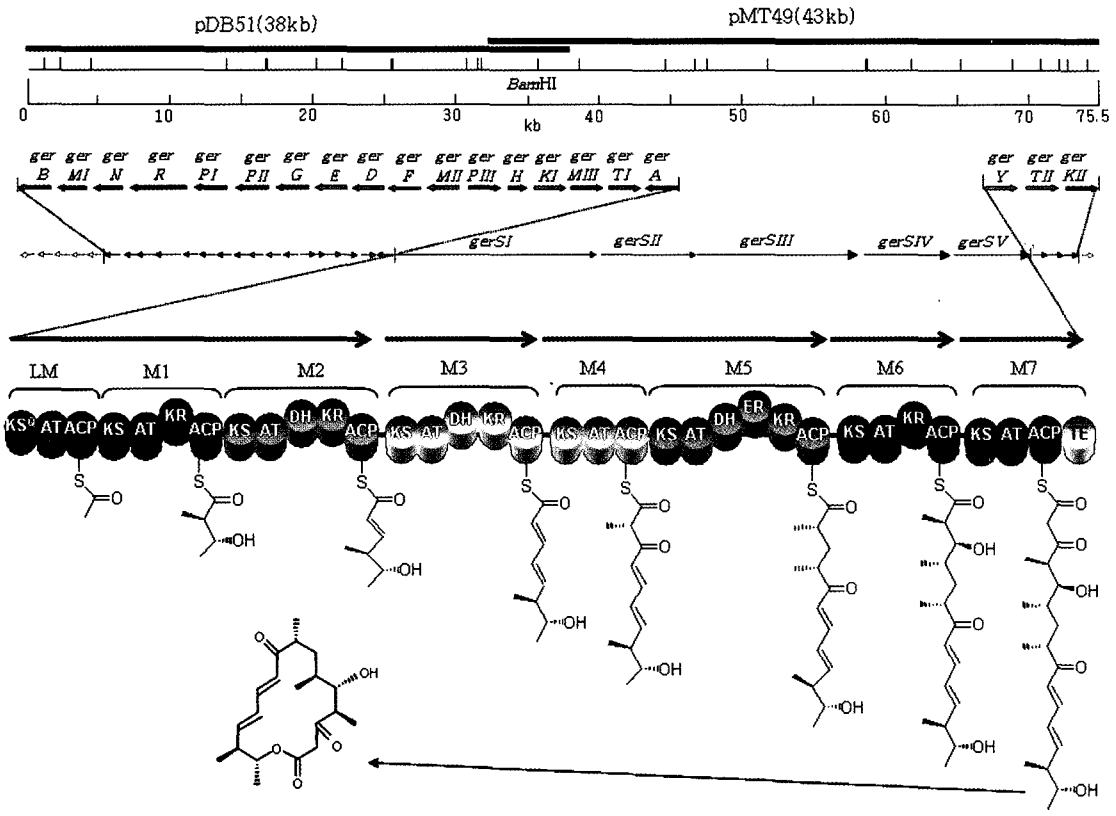

Fig. 1. The organization of the gene cluster for dihydrochalcomycin biosynthesis.
The arrows indicate the direction of transcription and relative size of the ORFs, deduced from analysis of the nucleotide sequence. Blue arrows indicate the genes involved in polyketide synthesis; green arrows, the genes involved in deoxysugar biosynthesis and transfer; brown arrows, the post-PKS genes; and black arrows, the resistance genes. The functions encoded by the ORFs are shown in Table 1.

and 2 in its resistance for the producer organism by homology search (Table 1). The remaining 6 ORFs (*ger1-ger5* and *ger31*) did not show any similarity with gene products that are known to be involved either in macrolide or in deoxysugar biosynthesis, which implies that those genes are unlikely to participate in dihydrochalcomycin biosynthesis. Even though *S.* sp. KCTC-0041BP isolated from Korean soil differs phylogenetically from *S. bikiniensis* based on 16S rRNA sequence (accession No. AM117492), the gene organization from *gerB* to *gerKII* involved in the biosynthesis was almost the same as that from *chmCIV* to *chmU* for the chalcomycin biosynthesis [35].

Each of the 5 large ORFs (*gerSI-gerSV*) located at the core of the dihydrochalcomycin gene cluster was found to encode a multifunctional type I PKS. The 5 giant PKS's contained 7 modules along with a loading domain, as anticipated. These modules catalyze 7 cycles of condensation reaction for chain elongation to give a 16-membered macrolide ring of dihydrochalcomycin [3]. The loading module contained a KS^Q domain, indicating that polyketide chain formation begins with a malonyl-CoA followed by its decarboxylation [36]. Besides this, all other KS domains contained the consensus DTACSSSLV motif with cysteine as an active residue [30]. The KR domain in module 4 was found to be inactive, since it completely lacks the consensus NADP(H)-binding motif, GxGxxGxxxA [14]. Furthermore, an ER domain required for the reduction of C10-C11 double bond was not found in the expected position in module 3. Similarly, the DH and KR domains in module 7 required for the formation of 2,3-*trans* double bond were absent. There is a possibility that these domains are present as discrete enzymes away from the dihydrochalcomycin gene cluster and act on the macrolide ring after its cyclization. Discrete KR and AT activities have already been reported in type I PKS-catalyzed reactions [5, 31].

The genes for the biosynthesis of two deoxysugars, D-chalcose and D-mycinose, in dihydrochalcomycin were also found in the cloned gene cluster. Based on the amino acid sequence similarity of the enzymes encoded by these genes with known enzymes involved in deoxysugar biosynthesis in other polyketides, a possible biosynthetic pathway is proposed (Fig. 2) and the function of each enzyme is suggested (Table 1, Fig. 2). Briefly, GerD and GerE are common to the biosynthesis of both deoxysugars and give dTDP-4-keto-6-deoxyglucose as a common intermediate, as reported previously [21, 22]. D-Mycinose

Table 1. Deduced functions of ORFs in the dihydrochalcomycin biosynthetic gene cluster.

Polypeptide	Amino acids (no.)	Closest homology[a]	% Identity[b]	Proposed function based on sequence similarity
Ger1	512	(*Ralstonia solanacerum*)	59	Putative transferase protein
Ger2	684	(*Ralstonia solanacerum*)	35	Hypothetical protein
Ger3	331	(*Ralstonia solanacerum*)	33	Hypothetical protein
Ger4	334	(*Mycobacterium tuberculosis H37Rv*)	41	Conserved hypothetical protein
GerO	334	Orf3 (*Streptomyces bikiniensis*)	87	Putative oxidoreductase
GerB	405	ChmCIV (*Streptomyces bikiniensis*)	93	3,4-Dehydratase-like protein
GerMI	271	ChmCI (*Streptomyces bikiniensis*))	87	Sugar-O-methyltransferase
GerN	485	ChmCV (*Streptomyces bikiniensis*))	88	NDP-4,6-dideoxyhexose-3,4-enoyl reductase
GerR	823	ChmR (*Streptomyces bikiniensis*))	90	Beta-glucosidase
GerPI	401	ChmPII (*Streptomyces bikiniensis*)	83	Cytochrome P-450: C8 hydroxylase
GerPII	407	ChmPI (*Streptomyces bikiniensis*)	93	Cytochrome P-450: C12-C13 epoxidase
GerG	274	ChmI (*Streptomyces bikiniensis*))	89	Type II thioesterase
GerE	323	ChmAII (*Streptomyces bikiniensis*))	96	dTDP-Glucose-4,6-dehydratase
GerD	295	ChmAI (*Streptomyces bikiniensis*)	98	Alpha(α)-D-glucose-1-phosphate thymidylyltransferase
GerF	196	ChmJ (*Streptomyces bikiniensis*)	95	NDP-hexose-3-epimerase
GerMII	255	ChmF (*Streptomyces bikiniensis*)	95	3-*O*-methyltransferase
GerPIII	420	ChmHI (*Streptomyces bikiniensis*)	89	Cytochrome P-450: C20 hydroxylase
GerH	073	ChmHII (*Streptomyces bikiniensis*)	81	Ferredoxin
GerKI	326	ChmD (*Streptomyces bikiniensis*)	92	Hexose-4-ketoreductase
GerMIII	403	ChmE (*Streptomyces bikiniensis*)	94	*O*-Methyltransferase
GerTI	418	ChmN (*Streptomyces bikiniensis*)	95	6-Deoxy-D-allosyltranferase
GerA	280	ChmB (*Streptomyces bikiniensis*)	91	23S rRNA transferase
GerSI	4,387	ChmGI (*Streptomyces bikiniensis*)	87	PKS [LM (KS^Q,AT, ACP),M1(KS,AT,KR,ACP),M2(KS,AT,DH,KR,ACP)]
GerSII	1,976	ChmGII (*Streptomyces bikiniensis*)	86	PKS [M3 (KS,AT,DH,KR,ACP)]
GerSIII	3,734	ChmGIII (*Streptomyces bikiniensis*)	86	PKS [M4 (KS,AT,KR*,ACP), M5 (KS,AT,DH,ER,KR,ACP)]
GerSIV	1,618	ChmGIV (*Streptomyces bikiniensis*)	88	PKS [M6 (KS,AT,KR,ACP)]
GerSV	1,357	ChmGV (*Streptomyces bikiniensis*)	89	PKS [M7 (KS,AT,ACP), TE]
GerY	404	ChmCII (*Streptomyces bikiniensis*)	84	NDP-hexose-3,4- isomerase
GerTII	425	ChmCIII (*Streptomyces bikiniensis*)	93	Chalcosyltransferase
GerKII	248	ChmU (*Streptomyces bikiniensis*)	90	3-Oxoacyl-(acyl-carrier-protein)-reductase (Post-PKS reductase)
Ger31	580	OlmRI (*Streptomyces avermitilis*)	35	Transcriptional regulator

ACP, acyl carrier protein; AT, acyltransferase; DH, dehydratase; ER, enoyl reductase; KR, β-ketoacyl-ACP reductase; KR*, an inactive KR; KS, β-ketoacyl-ACP synthase; KS^Q, a KS-like malonyl decarboxylase.
[a,b]Determined by BLAST search analyses.

biosynthesis in dihydrochalcomycin essentially follows the same pathway as found in the biosynthesis of this sugar in tylosin [2, 29]. For D-chalcose biosynthesis, the first intermediate is possibly a 3,4-isomerization followed by 3,4-dehydration and 3,4-enoylreduction to give dTDP-3-keto-4,6-dideoxyglucose. The candidate genes encoding these functions are *gerY*, *gerB*, and *gerN*, based on their homology with genes known to perform similar functions in other deoxysugar biosyntheses. The later product should be converted to a 3'-hydroxy derivative, but the potential enzyme catalyzing any such conversion was not found in the cluster. dTDP-4,6-dideoxyglucose is then attached to a macrolide ring by GerTII, and *O*-methylation by GerM1, similar to that by SpnH in spinosyn A [34], should occur only after glycosylation.

Similar to all other antibiotic producers, *S.* sp. KCTC-0041BP possesses genes that confer self-protection against the antibiotics it produces [8]. In the dihydrochalcomycin gene cluster, the *gerA* gene found immediate upstream of the PKS genes showed high similarity with the *tlr*B gene in the tylosin biosynthetic gene cluster in *S. fradiae*. TlrB methylates G748 in domain II of the 23s rRNA to inhibit the binding of tylosin to host ribosomes [23, 33]. However, no Erm type of methyltransferase, a major feature of MLS_B type of resistance, was found in the cloned gene cluster. Therefore, *gerA* is proposed as the only gene that provides primary self-resistance to this strain. Another gene product, GerR, showed high homology with OleR, a β-glucosidase in the olendomycin gene cluster in *S. antibioticus*, which reactivates oleandomycin extracelluarly [26, 37]. GerR is believed to play a similar role for dihydrochalcomycin. However, no homologue of OleI, which converts active oleandomycin into inactive oleandomycin glycoside [26], was found in the cloned dihydrochalcomycin gene cluster.

Four gene products in the dihydrochalcomycin gene cluster, GerPI-GerPIII and GerH, were found to be involved in post-PKS modification steps, based on their homology with other known enzymes [27]. GerPIII and GerH,

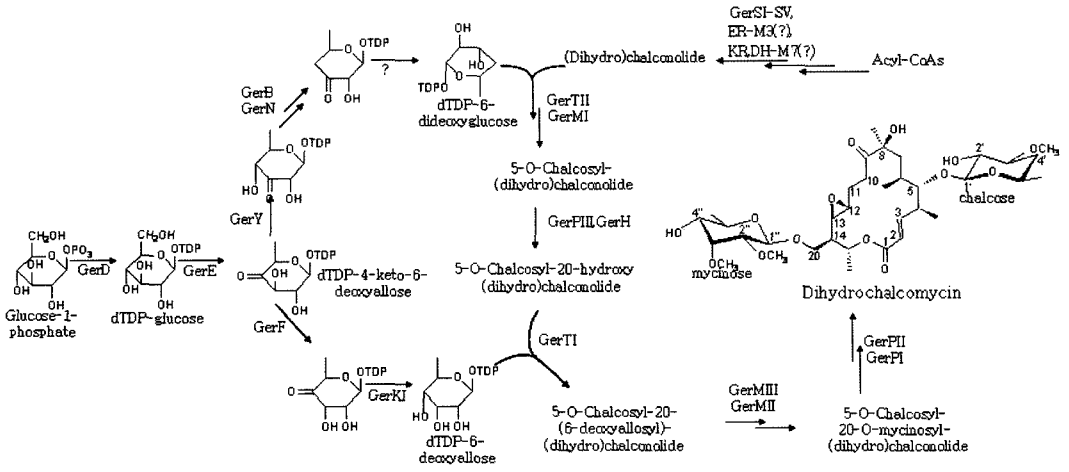

Fig. 2. Proposed biosynthetic pathway for dihydrochalcomycin in *Streptomyces* sp. KCTC-0041BP.

homologues of TylHI and TylI, respectively, should catalyze the hydroxylation of the CH_3-group into the CH_2-OH group at the C-20 position of the macrolide ring immediately after cyclization. GerPI, like its homologue LnmZ from the leinamycin gene cluster [31], should act as a hydroxylase at C-8. GerPII, which resembles OleP from the oleandomycin gene cluster, may catalyze epoxidation at the C-12-C-13 position. The enzyme GerG showed high homology with many type II TEs and is, therefore, likely to play an editing function in the biosynthesis by removing any misprimed starter or

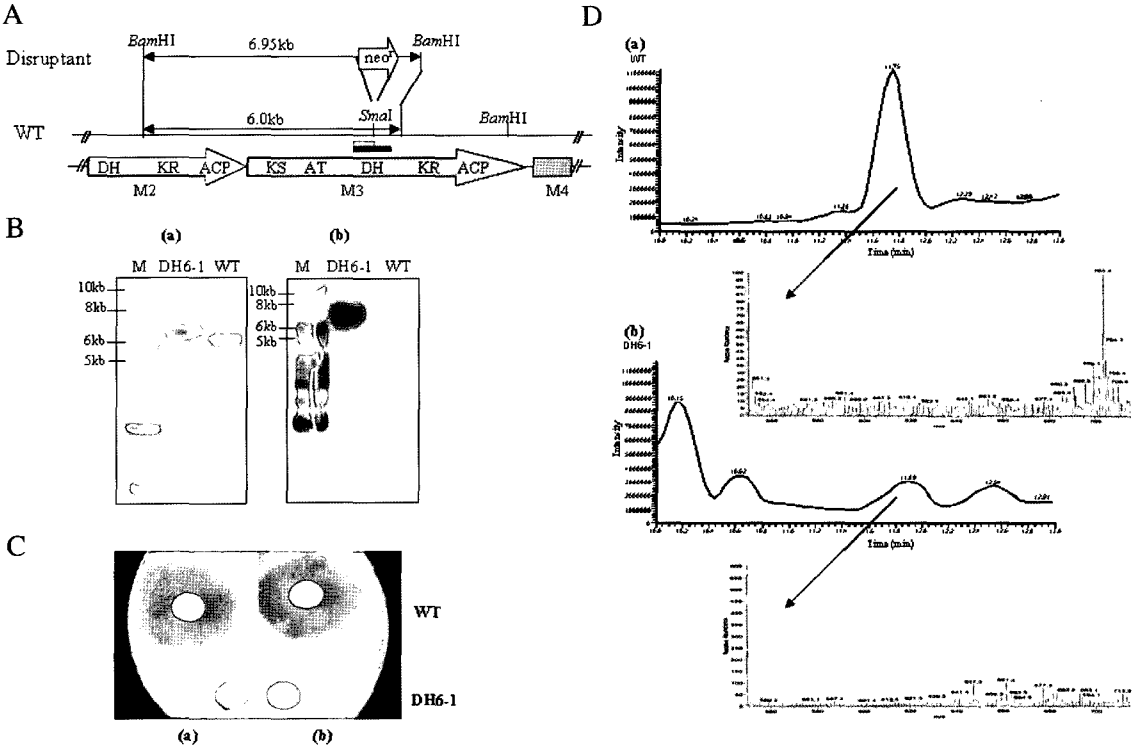

Fig. 3. Gene disruption of the DH domain in module 3 of the dihydrochalcomycin PKS system.

A. Predicted restriction enzyme polymorphism caused by gene disruption. The amplified region for the gene disruption is shown as a grey bar, and DH-3 probe is shown by a black bar. **B.** Southern hybridization of BamHI-digested chromosomal DNA of the wild-type (WT) strain and DH6-1 disruptant with DH-3 probe (a) and *neo'* probe (b). **C.** Antibacterial activity of ethyl acetate extract of WT strain and DH6-1 disruptant against *Micrococcus luteus* ATCC 9341 (a) and *Pseudomonas aeruginosa* GN T113 (b) as test organisms. **D.** LC chromatograms and MS (ESI) spectra for the production of dihydrochalcomycin in the WT (a) and DH6-1(b) strain of *Streptomyces* sp. KCTC-0041BP. The arrow indicates the peak for dihyrochalcomycin.

extender unit during the polyketide chain elongation reaction [18]. The function of GerO and GerK2 is not obvious in dihydrochalcomycin biosynthesis.

Confirmation of the Function of the Cloned PKS Genes in Dihydrochalcomycin Biosynthesis

A polyketide producer often possesses more than one PKS gene that may encode for different polyketides. Therefore, to confirm the involvement of the cloned PKS gene cluster in dihydrochalcomycin biosynthesis, the DH-3 domain in *gerSII* was disrupted by homologous recombination. Two exoconjugants, DH6-1 and DH6-2, were identified as double cross-over disruptants by their neomycin resistance and apramycin-sensitive phenotype. When the ethyl acetate extract of the culture broth was tested for antibacterial activity against *Micrococcus luteus* ATCC 9341 and *Pseudomonas aeruginosa* GN-T113, the extract of DH6-1 disruptant did not show any growth inhibition of the organisms tested, whereas that of WT strain showed a large inhibition zone (Fig. 3). Southern hybridization of the BamHI-digested chromosomal DNA with DH-3 probe showed a band at 6 kb in the WT and at 6.95 kb in DH6-1. The latter was due to the insertion of a 0.95-kb *neo*^{r} gene into the chromosomal DNA. As expected, the hybridization with the *neo*^{r} gene probe yielded the same 6.95 kb band in DH6-1, but no band in the WT. These results indicate that the *neo*^{r} gene was inserted into the correct locus at the DH-3 domain on the chromosome.

The LC-MS analyses of ethyl acetate extract dissolved in methanol revealed the $(M+H)^+$ peak of m/z 703.4, corresponding to dihydrochalcomycin, only in WT, but not in the disruptant DH6-1. The loss of chalcomycin production [m/z 701, $(M+H)^+$] along with dihydrochalcomycin in the disruptant was also confirmed by LC-MS analysis (data not shown). This implies that the PKS gene cluster for dihydrochalcomycin biosynthesis is simultaneously responsible for chalcomycin biosynthesis. Therefore, the proposed discrete PKS-related ER for the conversion of chalcomycin into dihydrochalcomycin, which is essential for C-10C-11 double-bond formation, might be partially active.

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REFERENCES

1. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipmann. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215:** 403–410.
2. Bate, N. and E. Cundliffe. 1999. The mycinose-biosynthetic genes of *Streptomyces fradiae*, producer of tylosin. *J. Ind. Microbiol. Biotechnol.* **23:** 118–122.
3. Bentley, R. and J. W. Bennett. 1999. Constructing polyketides: From colie to combinatorial biosynthesis. *Annu. Rev. Microbiol.* **53:** 411–446.
4. Bierman, M., R. Logan, K. O'Brien, E. T. Seno, R. N. Rao, and B. E. Schoner. 1992. Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene* **116:** 43–49.
5. Burgett, S. G., R. N. Kuhstoss, R. N. Rao, M. A. Richardson, and P. R. Rosteck, Jr. 1999. Platenolide synthase gene. *US Patent* 5,945,320.
6. Bycroft, M., K. J. Weissman, J. Stauton, and P. F. Leadlay. 2000. Efficient purification and kinetic characterization of a bimodular derivative of the erythromycin polyketide synthase. *Eur. J. Biochem.* **267:** 520–526.
7. Cocito, C., M. Di Giambattista, E. Nyssen, and P. Vannuffel. 1997. Inhibition of protein synthesis by streptogramins and related antibiotics. *J. Antimicrob. Chemother.* **39(Suppl. A):** 7–13.
8. Cundliffe, E. 1989. How antibiotic-producing organisms avoid suicide. *Annu. Rev. Microbiol.* **43:** 207–233.
9. Decker, H., S. Gaisser, S. Pelzer, P. Scheinder, L. Westrich, W. Wohlben, and A. Bechthold. 1996. A general approach for cloning and characterizing dNDP-glucose dehydratase genes from actinomycetes. *FEMS Microbiol. Lett.* **141:** 195–201.
10. Dennis, F. and R. Brzezinski. 1991. An improved aminoglycoside resistance gene cassette for use in Gram-negative bacteria and *Streptomyces*. *FEMS Microbiol. Lett.* **81:** 261–264.
11. Hopwood, D. A. and D. H. Sherman. 1990. Molecular genetics of polyketides and its comparison to fatty acid biosynthesis. *Annu. Rev. Genet.* **24:** 37–66.
12. Ishikawa, J. and K. Hotta. 1999. Frame plot: A new implementation of the frame analysis for predicting protein-coding regions in bacterial DNA with high G+C content. *FEMS Microbiol. Lett.* **174:** 251–253.
13. Izumikawa, M., M. Murata, K. Tachibanqa, Y. Ebizuka, and I. Fujii. 2003. Cloning of modular type I polyketide synthase genes from salinomycin producing strain of *Streptomyces albus*. *Bioorg. Med. Chem.* **11:** 3401–3405.
14. Kakavas, S. J., L. Katz, and D. Stassi. 1997. Identification and characterization of the niddamycin polyketide synthase genes from *Streptomyces caelestis*. *J. Bacteriol.* **179:** 7515–7522.
15. Katz, L. and S. Donadio. 1993. Polyketide synthesis: Prospects for hybrid antibiotics. *Annu. Rev. Microbiol.* **47:** 875–912.
16. Khosla, C. and R. J. X. Zaweda. 1996. Generation of polyketide libraries via combinatorial biosynthesis. *TIBTECH* **14:** 335–341.
17. Kieser, T., M. J. Bibb, M. J. Buttner, K. F. Chater, and D. A. Hopwood. 2000. *Practical Streptomyces Genetics*. John Innes Centre, Norwich, England.
18. Kim, B. K., T. A. Cropp, B. J. Beck, D. H. Sherman, and K. A. Reynolds. 2002. Biochemical evidence for an editing

role of thioesterase II in the biosynthesis of the polyketide pikromycin. *J. Biol. Chem.* **277:** 48028–48034.
19. Kim, S. D., I. J. Ryoo, C. J. Kim, W. G. Kim, J. P. Kim, J. Y. Kong, H. Koshino, M. Uramoto, and I. D. Yoo. 1996. GERI-155, a new macrolide antibiotic related to chalcomycin. *J. Antibiot.* **49:** 955–957.
20. Ko, H.-R., H. Kakeya, A. Yoshida, R. Onose, M. Ukei, M. Muroi, A. Takatsuki, H. Matsuzaki, and H. Osada. 2002. PC-766B' and PC-766B, 16-membered macrolide angiogenesis inhibitors produced by *Norcadia* sp. RK97-56. *J. Microbiol. Biotechnol.* **12:** 829–833.
21. Lee, H., J. K. Sohng, H. J. Kim, D. H. Nam, J. M. Han, S. S Cho, J. H. Choi, and J. C. Yoo. 2004. Cloning and expression of the glucose-1-phosphate thymidylyltransferase gene (*ger*D) from *Streptomyces* sp. GERI-155. *Mol. Cells* **17:** 274–280.
22. Lee, H., J. Sohng, H. Kim, D. Nam, C. Seong, J. Han, and J. Yoo. 2004. Cloning, expression and biochemical characterization of dTDP-glucose 4,6-dehydratase (*ger*E) from *Streptomyces* sp. GERI-155. *J. Microbiol. Biotechnol.* **14:** 576–583.
23. Liu, M., F. Kirpekar, G. P. van Wezel, and S. Douthwaite. 2000. The tylosin resistance gene *tlrB* of *Streptomyces fradiae* encodes a methyltransferase that targets G748 in 23s rRNA. *Mol. Microbiol.* **37:** 811–820.
24. MacNeil, D. J., K. M. Gewain, C. L. Ruby, G. Dezeny, P. H. Gibbons, and T. MacNeil. 1992. Analysis of *Streptomyces avermitilis* genes required for avermectin biosynthesis utilizing a novel integration vector. *Gene* **111:** 61–68.
25. O'Hagan, D. 1995. *Evolution of the Polyketide Metabolites.* Ellis Horwood , Chichester, U.K.
26. Quirós, L. M., I. Aguirrezabalaga, C. Olano, C. Méndez, and J. A. Salas. 1998. Two glycosyltransferase and a glucosidase are involved in oleandomycin modification during its biosynthesis by *Steptomcyces antibioticus*. *Mol. Microbiol.* **28:** 1177–1185.
27. Rix, U., C. Fischer, L. L. Remsing, and J. Rohr. 2002. Modification of post-PKS tailoring steps through combinatorial biosynthesis. *Nat. Prod. Rep.* **19:** 542–580.
28. Sambrook, J. and D. W. Russell. 2001. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
29. Sohng, J. K., H. J. Kim, D. H. Nam, D. O. Lim, J. M. Han, H. J. Lee, and J. C. Yoo. 2004. Cloning, expression and biological function of dTDP-deoxyglucose epimerase (*ger*F) gene from *Streptomyces* sp. GERI-155. *Biotechnol. Lett.* **26:** 185–191.
30. Tang, L., Y. J. Yoon, C. Y. Choi, and C. R. Hutchinson. 1998. Characterization of the enzymatic domains in the modular polyketide synthase involved in rifamycin B biosynthesis by *Amycolaptosis mediterranei*. *Gene* **216:** 255–265.
31. Tang, G.-L., Y.-Q. Cheng, and B. Shen. 2004. Leinamycin biosynthesis revealing unprecedented architectural complexity for a hybrid polyketide synthase and nonribosomal peptide synthase. *Chem. Biol.* **11:** 33–45.
32. Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22:** 4673–4680.
33. Vester, B. and S. Douthwaite. 2001. Macrolide resistance conferred by base substitutions in 23S rRNA. *Antimicrob. Agents Chemother.* **45:** 1–12.
34. Waldron, C., P. Matsushima, P. R. Rosteck, Jr., M. C. Broughton, J. Turner, K. Madduri, K. P. Crawford, D. J. Merlo, and R. H. Baltz. 2001. Cloning and analysis of the spinosad biosynthetic gene cluster of *Saccharopolyspora spinosa*. *Chem. Biol.* **8:** 487–499.
35. Ward, S. L., Z. Hu, A. Schirmer, R. Reid, W. P. Revill, C. D. Reeves, O. V. Petrakovsky, S. D. Dong, and L. Katz. 2004. Chalcomycin biosynthesis gene cluster from *Streptomyces bikiniensis*: Novel features of an unusual ketolide produced through expression of the *chm* polyketide synthase in *Streptomyces fradiae*. *Antimicrob. Agents Chemother.* **78:** 4703–4712.
36. Witkwoski, A., A. K. Joshi, Y. Lindqvist, and S. Smith. 1999. Conversion of a β-ketoacyl synthase to a malonyl decarboxylase by replacement of the active-site cysteine with glutamine. *Biochemistry* **38:** 11643–11650.
37. Zhao, L., N. L. Beyer, S. A. Borisova, and H. Liu. 2003. β-Glycosylation as a part of self-resistance mechanism in methymycin/pikromycin producing strain *Streptomyces venezuelae*. *Biochemistry* **42:** 14794–14804.