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Method for Cloning Biosynthetic Genes of Secondary Metabolites Including Deoxysugar from Actinomycetes

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Many antibiotics contain partially deoxygenated sugar components that are usually essential for biological activity, affinity, structural stability, and solubility of antibiotics. Gene probes of the biosynthetic genes related with the deoxysugar were obtained from PCR. Primers were designed from the conserved peptide sequences of the known dTDP-D-glucose 4,6-dehydratases, which are the key step enzymes in the biosynthesis of deoxysugar. The primers were applied to amplify parts of dehydratase genes to 27 actinomycetes that produce the metabolites containing deoxysugar as structural constituents. About 180 and 340 bp DNA fragments from all of the actinomycetes were produced by PCR and analyzed by Southern blot and DNA sequencing. The PCR products were used as gene probes to clone the biosynthetic gene clusters for the antibiotic mithramycin, rubradirin, spectinomycin, and elaiophyrin. This method should allow for detecting of the biosynthetic gene clusters of a vast array of secondary metabolites isolated from actinomycetes because of the widespread existence of deoxysugar constituents in secondary metabolites.

Keywords: Actinomycete, Deoxysugar, dTDP-D-glucose 4,6-dehydratase, PCR, Secondary metabolites.

Introduction

Actinomycetes are the representative microbial group sources for a variety of secondary metabolites. Almost all of bioactive molecules are the end products produced through complex multistep biosynthetic pathways. Recent progresses from biochemical and genetic studies on the biosynthetic pathways of secondary metabolites led to redesign these pathways in a directed fashion to produce hybrid type metabolites and to overproduce the secondary metabolites (Khosla *et al.*, 1993; Meurer *et al.*, 1997). It has been demonstrated from studies on more than 100 pathways in actinomycetes that the biosynthesis genes of secondary metabolites are clustered. Since secondary metabolites are produced through several multistep pathways, DNA gene clusters encoding biosynthetic genes range from 20 to 200 kb (Pissowotzke *et al.*, 1991; Weigh and Bibb, 1992). Many secondary metabolites, including macrolides, anthracyclines (Fujiwara *et al.*, 1983), polyenes, polyethers, and polypeptide, have glycone moieties (Fig. 1). Most glycones are deoxysugars: 6-deoxysugar, dideoxysugar, aminodeoxysugar, nitrodeoxysugar, and trideoxysugar (Bechthold *et al.*, 1995; Madduri *et al.*, 1995; Sohng *et al.*, 1996). The key biosynthetic step of deoxysugars is the conversion of dTDP-D-glucose to dTDP-4-keto-6-deoxy-D-glucose by dTDP-D-glucose 4,6-dehydratase (Snipes *et al.*, 1979; Merson-Davies and Cundiffe, 1994). When the gene encoding the dNDP-D-glucose 4,6-dehydratase is cloned, the gene clusters flanking the dehydratase gene could be examined to identify the whole biosynthetic genes involved in secondary metabolites containing deoxysugars. This paper introduces a universal method to clone the biosynthetic gene clusters of secondary metabolites containing deoxysugar moieties.

Materials and Methods

Bacterial strains, plasmids, and growth conditions Actinomycetes used in this study are listed in Table 1. Plasmid-containing *Escherichia coli* strains were grown in Luria broth containing appropriate antibiotics. *S. achromogene* var. *rubradiris* NRRL3061 was obtained from ARS collection (Peoria, USA). *S. violaceoruber* Tü22 was obtained from Professor H. Zahner, Tübingen. *Streptomyces* sp. C5 was from the Frederick Cancer

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Table 1. The twenty-seven actinomycetes used in this study.

Strains	Antibiotic	Classification of Antibiotics	Deoxysugar Moiety
S. achromogenes	Rubradirin	Ansamycin	Rubranitrose
S. peucetius ATCC29050	Adriamycin	Anthracycline	Daunosamine
S. peucetius ATCC27952	Adriamycin	Anthracycline	Daunosamine
S. sp. C5	Adriamycin	Anthracycline	Daunosamine
S. spectabilis	Spectinomycin	Aminoglucoside	Dideoxysugar
Micromonospora saitamica	Rubradirin	Ansamycin	Rubranitrose
S. argillaceus KCC9016	Mithramycin	Tricyclic	Mycarose, Oliose, Olivose
S. galiaceus KCCM12456	Aclacinomycin	Anthracycline	Rhodosamine, Cinerulose, 2-Deoxy-L-fucose
S. bambergiensis KCC9019	Noenomycin	Anthracycline	Aminodideoxysugar
S. rimosus KCC1077	Rimosin	Macrolide	Dideoxysugar
S. sp. MCY-846	Elaiophylin	Macrolide	Dideoxysugar
S. niveus ATCC19753	Novobiotin	Coumarin	Deoxysugar
S. nogalator ATCC27451	Nogalamycin	Anthracycline	Deoxysugar
S. venezuelae ATCC15439	Neomethylmycin	Macrolide	Desosamine
S. sp. SCC-2136 ATCC19795	Sch 47554	Angucycline	Amicetose, Aculose
Actinomudura sp. ATCC39334	Esperamycin	Enediynee	Deoxysugar, Aminodideoxysugar
Amycolatopsis orientalis subsp. orientalis ATCC19759	Vancomycin	Polypeptide	Vacosamine
Actinomudura roseorufa	Octacylclomycin	Polyene	Trideoxysugar
S. thermotolerans ATCC11416	Carbomycin	Macrolide	Aminodideoxysugar
S. fradiae ATCC19609	Tyrosin	Macrolide	Mycinomycin, Mycinose, Mycarose
S. amvofaciens ATCC23877	Foromacidin	Macrolide	Dideoxysugar, Aminodideoxysugar Aminotetradeoxysugar
S. noursei KCC1083	Nystatin	Macrolide	Mycosamine
S. viridochromogenes KCC9009	Avilamycin	Oligoglycoside	Curacose, Evermicose, 2,6-Dideoxy-D-arabino-pyranose, Curancin
S. platensis KCCM12367	Platenomycin	Macrolide	Mycarose, Mycaminose
S. griseoflavus KCCM12624	Novobiocin	Coumarin	Deoxysugar
S. vioreceoruber Tü22	Granaticin	Tricyclic	Dideoxysugar
S. griseus	Streptomycin	Aminoglucoside	Streptose

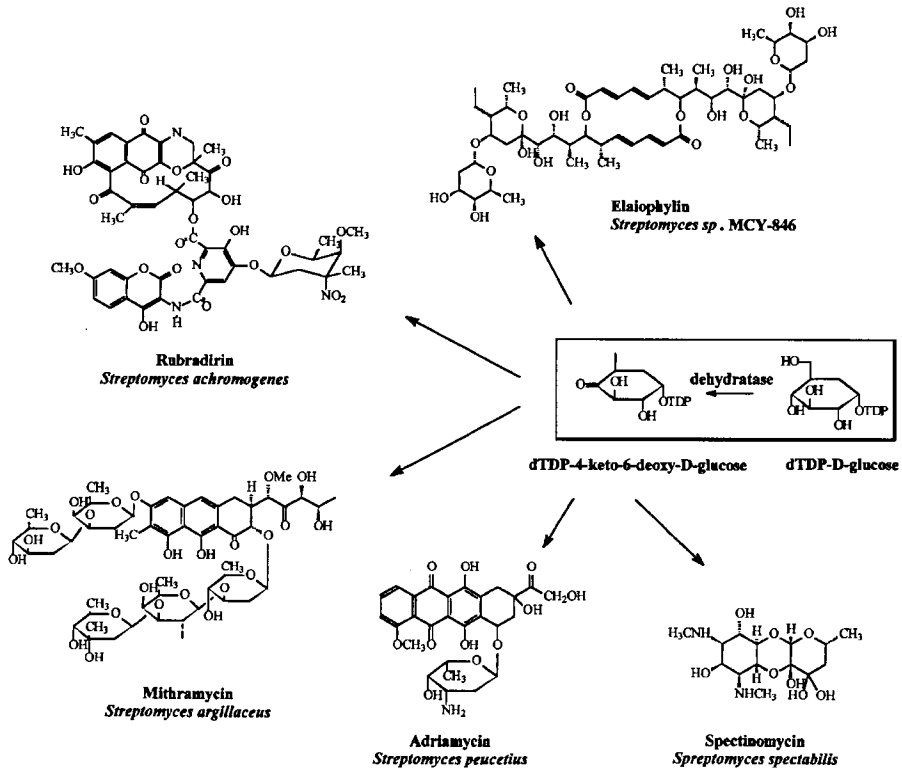

Fig. 1. Several secondary metabolites containing 6-deoxysugar.

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   81          Homologue-1                                    Homologue-2
1  LLDRILPGHD AVVHFAAESH VDRSLRSASE FVRTNVAGTQ TLLDACLAAG T.GR..FVHV STDEVYGSIA
2  LLDRVLPGHD AVVHFAAESH VDRSLTGPGE FVRTNVMGTQ QLLDAALHAG V.DR..VLHV STDEVYGSLD
3  WWRRLMEGVG LVVHFAAESH VDRSIESSEA FVRTNVEGTR VLLQAAVDAG V.GR..FVHI STDEVYGSIA
4  VVSEVMREVD VVVHFAAETH VDRSILGASD FVVTNVVGTN TLLQGALAAN V.SK..FVHV STDEVYGTIE
5  PGRRVMAGGD GVVHLAAESH VQRSILDASV FVRTNVHGTQ TLLDAATRHG VADRSSFVQV STDEVYGSLE

   151                                   Homologue-3
1  EGAWTEDHPL LPNSPYSASK ASSTCSSAPV TAH.HGLNVS ITRCSNNYGP HQHPEKLIPL FVTNLLEGLP
2  SGTWTEDSPL LPNSPYAASK ASTTWSAAPT TVR.HGLDVR ITRCSNNYGP RQHPEKLIPN FVTRLLTGRQ
3  EGSWPEDHPV APNSPYAATK AASDLLALAY HRT.YGLDVR VTRCSNNYGP RQYPEKAVPL FTTNLLDGLP
4  HGSWPEDHIL EPNSPYSAAK AGSDLIARAY HRT.HGLPVC ITRCSNNYGP YQFPEKVIPL FITNLNDGRR
5  HGSWTEDEPL RPNSPYSASK ASGDLLALAH HVHSHGLDVR VTRCSNNYGP RQFPEKLIPR FITLLMDGHR

PCR Primer: Homologue-1 (HFAAESHV) Sence
DW-1: 5'-CACTTCGGYGGYGAGTCYCACGT-3'
PCR Primer: Homologue-2 (STDEVYG) Sence
DW-2: 5'-TCYACYGACGAGGTYTACCG-3'
PCR Primer: Homologue-3 (CSNNTGP) Antisence
DW-3: 5'-GGYCCGTAGTTGTTYGAGCA-3'
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Fig. 2. Amino acid alignments of the known dehydratases. (1) OxiI, from *S. antibioticus* Tü99 (Sohng *et al.*, 1996), (2) GraE, from *S. violaceoruber* Tü22 (Bechthold *et al.*, 1995), (3) Orf4, from *S. fradiae* T59235 (Krügel *et al.*, 1993), (4) Orf2, from *Saccharopolyspora erythraea* (Pissowotzke *et al.*, 1991), and (5) StrE, from *S. griseus* (Pissowotzke *et al.*, 1991).

Research Center, Frederick, USA. Other actinomycetes were purchased from ATCC (American Type Culture Collection, Rockville, USA), KCTC (Korean Collection for Type Cultures, Daejeon, Korea) and KCCM (Korean Culture Center of Microorganisms, Seoul, Korea). All actinomycetes were grown at 26°C on YMB (0.3% Bacto-yeast extract, 0.5% Bacto peptone, 0.3% Malt extract, 1% Glucose, and 0.2% Trace element). For the growth of actinomycetes on plates, R2YE medium was used. *S. antibioticus* Tü99 was grown at 30°C on Emerson plates (0.4% beef extract, 0.5% Bacto-yeast extract, 0.4% peptone, 1% dextrose, 0.5% NaCl, and 2% Bacto-agar) (Holzbach *et al.*, 1978). General culture methods of *Streptomyces* by the method of Hopwood *et al.* (1985) were followed.

Enzymes and chemicals Synthetic oligonucleotides were synthesized by Korea Biotec Inc. Polymerase chain reaction (PCR) was carried out with a PCR kit (Pre-Mix^{TM}-TOP, Korea Biotec Inc.). All other chemicals were obtained from Sigma (St. Louis, USA) or United States Biochemical (Cleveland, USA). Restriction enzymes, random labeling kit, and other enzymes were purchased from Promega Biotec. (Madison, USA).

Isolation of chromosomal DNA Genomic DNAs were prepared from mycelia collected from 50 ml cultures grown in YMB or TSB (1.7% Bacto tryptone, 0.3% Bacto soytone, 0.25% dextrose, 0.5% NaCl, and 0.25% K_2HPO_4) containing 0.5% glycine, 5 mM $MgCl_2$, and toothpicks. Mycelia were collected by centrifugation, washed twice with 10.3% sucrose, and resuspended in 6.2 ml of lysis buffer [15% sucrose, 25 mM Tris-HCl (pH 8.0) and 25 mM EDTA] containing 5–10 mg of lysozyme per ml. After 10 min of incubation at 37°C, protease solution was added in a final concentration of 0.5 mg/ml and the mixture was incubated at 70°C for 10 min. The mixture was then worked up in standard fashion (Hopwood *et al.*, 1985), and the genomic DNA was dissolved in 1 ml of sterile TE buffer.

Design of PCR primer PCR primers of dTDP-D-glucose 4,6-dehydratase gene were designed according to the amino acid consensus sequences found within the known five dehydratases. They showed 50 to 80% identity (Romana *et al.*, 1991; Marumo *et al.*, 1992; Thomson *et al.*, 1992; Torson *et al.*, 1993). In particular, three parts of the peptide showed highly conserved identity and simple genetic codon usage (Fig. 2). Based on the amino acid consensus sequences, three oligonucleotide primers (DW11, DW21, and DW32) were synthesized by taking into account the preferred codon usage of *Streptomyces* having 50% G or C in the first base, 75% in the second base, and 90% in the third base of each codon (Bibb *et al.*, 1984; Weigh and Bibb, 1992). DW11 was designed from homologue-1 site (sense, HFAAESHV: 5′-CAC TTC GGY GGY GAG TCY CAC GT-3′), DW21 from homologue-2 (sense, STDEVYG: 5′-TCY ACG GAC GAG GTC TAC GG-3′), and DW32 from homologue-3 (antisense, CSNNYGP: 5′-GGY CCG TAG TTG TTY GAG CA-3′, Y=G/C).

PCR amplification PCR reactions were carried out with the PCR kit (Pre-Mix^{TM}-TOP, Korea Biotec. Inc.) containing $MgCl_2$ and glycerol. Reaction conditions were as following: one cycle at 94°C for 5 min and 67°C for 1 min; 32 cycles at 94°C for 1 min, 67°C for 1 min, and 74°C for 1 min, with a 7 min extension at 74°C for the last cycle. PCR amplified DNAs were detected by agarose gel electrophoresis.

Sequence of PCR products The DNA sequences of the cloned 340 bp PCR products were determined by the dideoxy nucleotide chain termination method (Sanger *et al.*, 1977) using the PCR sequencing kit (Promega) and Sequence version 2.0 kit (United States Biochemicals Co.). Two primers (DW11 and DW32) were used to sequence each PCR product. The sequence data of the PCR products were analyzed by the SeqEdit Program.

Southern hybridization The PCR DNAs were subjected to electrophoresis on a 1.5% agarose gel. The DNA from the agarose gel was transferred to nitrocellulose membranes by capillary action. Prehybridization and overnight hybridization were conducted in the presence of 40% (v/v) formamide and 4% Blotto solution at 50°C. *chlE*, the dTDP-D-glucose 4,6-dehydratase gene from *S. antibioticus* Tü99, was labeled with [^{32}P]-dCTP using a random labeling kit. The membranes were washed with 2× SSC and 0.1% SDS at room temperature twice for 15 min each, and with 0.2× SSC and 0.1% SDS for 1 h at 50°C with gentle agitation. Autoradiography was carried out at −78°C for 3–4 h using X-Omat AR film in a cassette with intensifying screens (Sambrook *et al.*, 1989).

Results and Discussion

DNA sequence analysis of the PCR products The sizes of expected PCR products were 340 bp with primer-DW11 and primer-DW32 and 180 bp with primer-DW21 and primer-DW32. Using PCR, DNA fragments with sizes of 340 bp and 180 bp were obtained from the 27 actinomyces species, with the exception of *S. bambergiensis* for the 180 bp fragment (Fig. 3). The *chlE* probe was used to hybridize to the PCR products. The *chlE* is the gene of dTDP-D-glucose 4,6-dehydratase, cloned and expressed from *S. antibioticus* Tü99 (Sohng *et al.*, 1998). All the 180 bp and 340 bp DNA fragments showed strong bands (Fig. 4). These positive bands indicate high homogeneity between PCR products and dTDP-D-glucose 4,6-dehydratase gene. Four of the 340 bp PCR products obtained were sequenced to confirm that they were the dehydratase genes. The PCR products from *Streptomyces* sp. MCT846 (elaiophylin producer), *S. argil-laceus* (mithramycin producer), *S. spectabilis* (spectino-mycin producer), and *S. achromogenes* var. *rubradiris* NRRL3061 (rubradirin producer) were selected for DNA sequencing. Their deduced amino acid sequences were compared with the known dehydratases and showed 60 to 95% identity (Fig. 5). This result indicates that the PCR products are really the gene encoding the dTDP-D-glucose 4,6-dehydratase. Therefore, DW11, DW21, and DW32 can be used as general primers to prepare the dehydratase gene probe from actinomycetes which produce secondary metabolites containing deoxysugar moieties.

Analysis of the genes flanking the isolated dehydratase gene In order to demonstrate that the genes flanking the isolated dehydratase genes are related with the biosynthesis of metabolites containing deoxysugars, the flanking genes

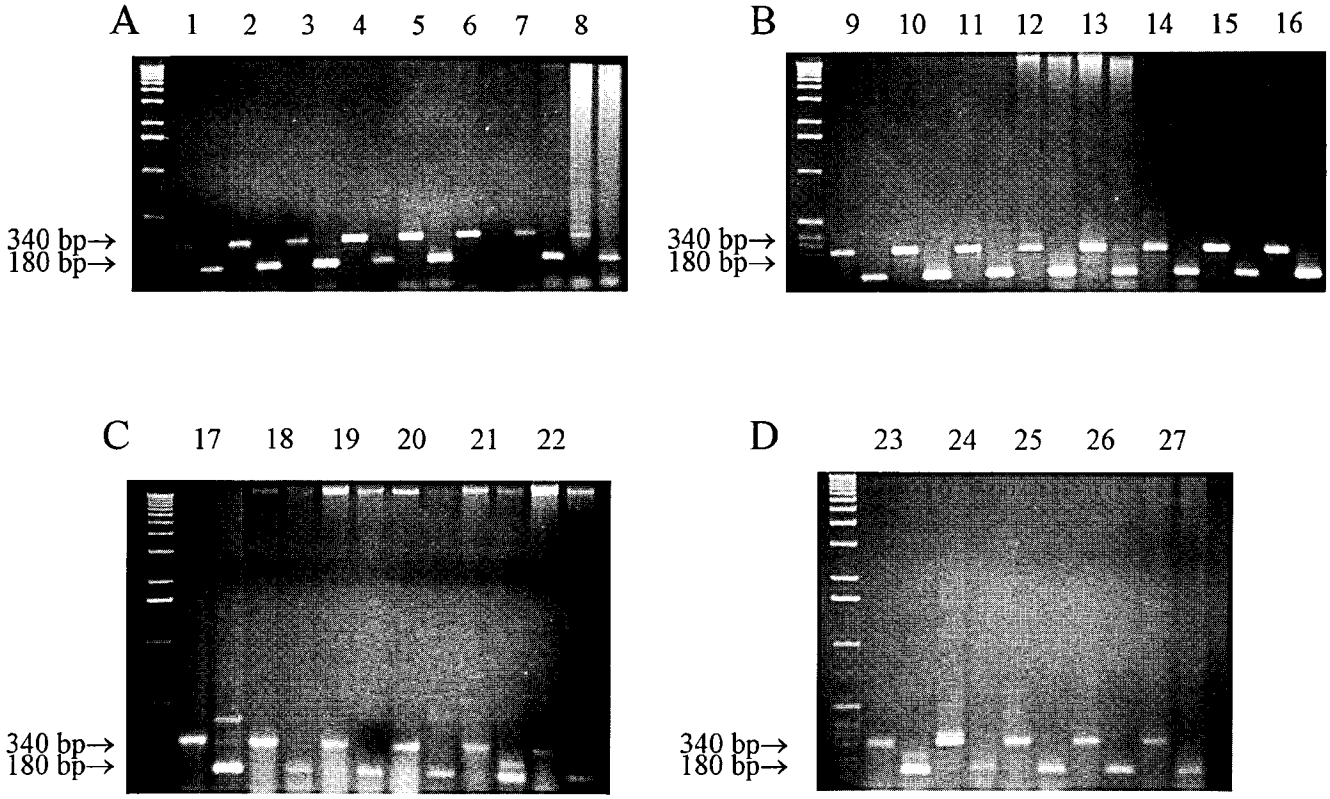

Fig. 3. PCR products from 27 actinomycetes. A. Lane 1, 340 bp and 180 bp of *S. achromogenes* (Bhuyan *et al.*, 1965); lane 2, *S. peucetius* (ATCC29050) (Thompson *et al.*, 1992); lane 3, *S. peucetius* (ATCC27952) (Hong *et al.*, 1994); lane 4, *S.* sp. C5 (Thompson *et al.*, 1992); lane 5, *S. spectabilis* (Price, 1986); lane 6, *S. argillaceus* (Grundy *et al.*, 1995); lane 7, *S. bambergiensis* (Soldatova *et al.*, 1994); lane 8, *S. rimosus* (Martern *et al.*, 1973). B. Lane 9, *S. galiaceus* (Kantola *et al.*, 1997); lane 10, *S. noursei* (Mitrofanova *et al.*, 1991); lane 11, *Micromonospora saitamica*; lane 12, *S. viridochromogenes* (Gaisser *et al.*, 1997); lane 13, *S. platensis* (Furumai and Suzuki, 1975); lane 14, *S. griseoflavus* (Feng, 1995); lane 15, *S. vioreceoruber* Tü22 (Bechthold *et al.*, 1995); lane 16, *S. griseus* (Pissowotzki *et al.*, 1991; Distler *et al.*, 1992). C. Lane 17, *S. sp.* MCT846 (Lee *et al.*, 1996); lane 18, *S. niveus* (Hoggarth and Ritchie, 1995); lane 19, *S. nogalator* (Wiley *et al.*, 1977); lane 20, *S. venezuelae* (Facey *et al.*, 1996); lane 21, *S.* sp. SCC-2136 (Chu *et al.*, 1993); lane 22, *Actinomadura* sp. D. Lane 23, *Amycolatopsis orienthalis* subsp. *Orientalis* (Sheldrick *et al.*, 1978); lane 24, *Actinomudura roseorufa* (Funayama *et al.*, 1992); lane 25, *S. thermotolerans* (Arisawa *et al.*, 1995); lane 26, *S. fradiae* (Krügel *et al.*, 1996); lane 27, *S. amvofaciens* (Malet *et al.*, 1992).

were analyzed by Southern blot and random sequencing. Cosmid DNA libraries for *S. argillaceus*, *S. achromogenes*, *S. spectibilis* and *Streptomyces* sp. MCT846 were constructed. The 340 bp PCR products were used as gene probes to screen cosmid clones from the DNA libraries by colony hybridization. Analysis of the flanking genes were done with *eryA* (the gene encoding the type I polyketide synthase), *actI* (the gene encoding the type II polyketide synthase), AHBA synthase gene (the gene involved in biosynthesis of 3-amino-5-hydroxybenzoic acid, a precursor of rifamycin), or *strD* (the gene encoding the glucose-1-phosphate thymidylyltransferase) as gene probes by Southern blot.

Mithramycin isolated from *S. argillaceous* (Grundy *et al.*, 1995; Felipe *et al.*, 1996) is a tricyclic polyketide containing D-olivose-L-mycarose and D-oliose-D-olivose-D-olivose as deoxysugar constituents. Two cosmid clones pLS14 and pLS15 were isolated from the *S. argillaceus* cosmid library. pLS15 digested with several restriction enzymes was bound strongly to the PCR DNA and the *actI* gene probe (Fig. 6). The dehydratase gene was located at about 10 kb apart from the genes encoding polyketide synthase (Fig. 7).

An ansamycin antibiotic rubradirin was isolated from *S. achromogenes* var. *rubradiris* NRRL 3061 (Bhuyan *et al.*, 1965; Meyer, 1965) and consists of four distinct moieties, rubransarol, 3-amino-4-hydroxycoumarin, dihydroxydipicolinic acid, and rubranitrose (Reusser, 1979). Screening of the cosmid library constructed from the *S. achromogenes* genomic DNA has led to the identification of three kinds of cosmid clones with unlinked DNA regions. One of the three kinds of cosmid clones was demonstrated by Southern blot analysis to contain the genes homologous to AHBA synthase and *eryA* genes. The size of the gene homologous to *eryA* is about 28 kb and the AHBA synthase gene homologue resides between the *eryA* homologous genes. The gene cluster identified is about 45 kb (Sohng *et al.*, 1997).

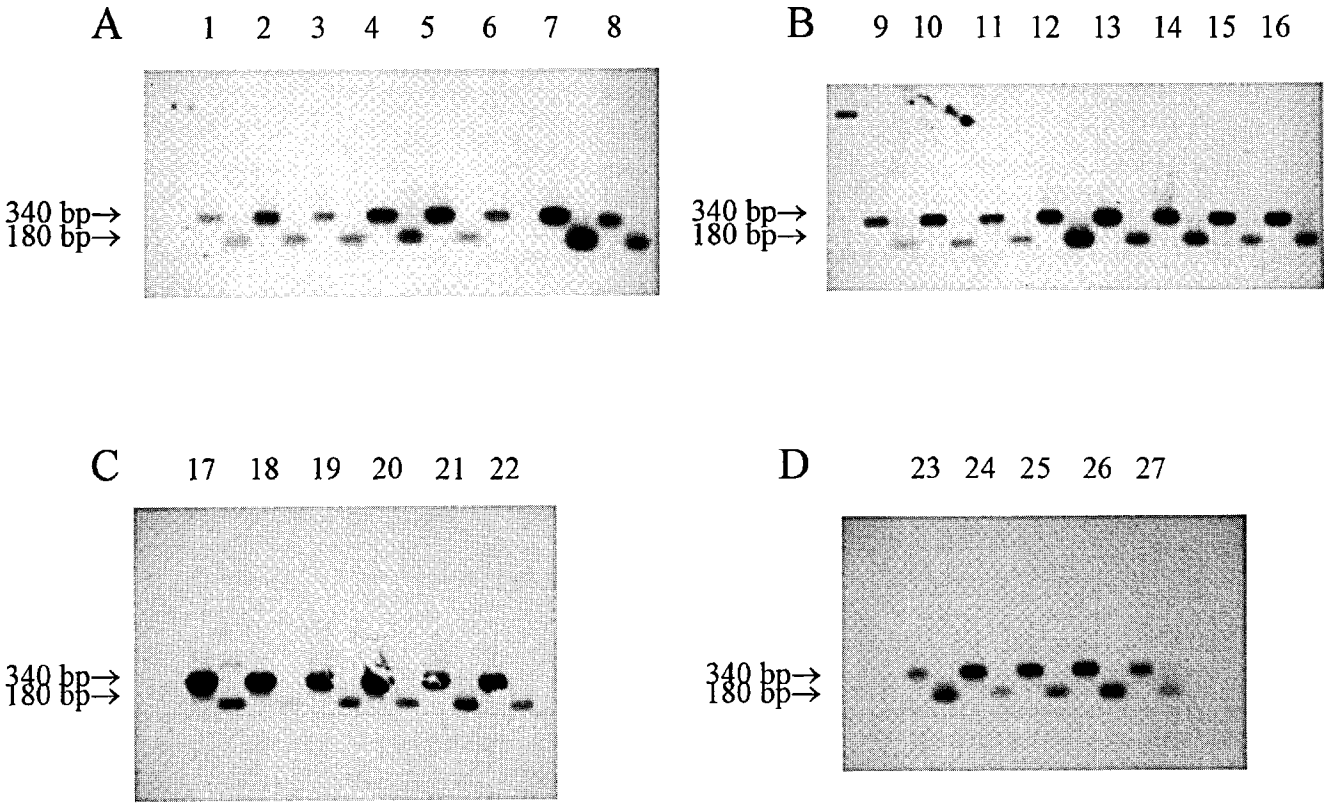

Fig. 4. Southern blot hybridization of the PCR products obtained from 27 *Actinomycetes* with ^{32}P-labeled *chlE*. A. Lane 1, 340 bp and 180 bp of *S. achromogenes* (Bhuyan *et al.*, 1965); lane 2, *S. peucetius* (ATCC29050) (Thompson *et al.*, 1992); lane 3, *S. peucetius* (ATCC27952) (Hong *et al.*, 1994); lane 4, *S.* sp. C5 (Thompson *et al.*, 1992); lane 5, *S. spectabilis* (Price, 1986); lane 6, *S. argillaceus* (Grundy *et al.*, 1995); lane 7, *S. bambergiensis* (Soldatova *et al.*, 1994); lane 8, *S. rimosus* (Martern *et al.*, 1973). B. Lane 9, *S. galiaceus* (Kantola *et al.*, 1997); lane 10, *S. noursei* (Mitrofanova *et al.*, 1991); lane 11, *Micromonospora saitamica*; lane 12, *S. viridochromogenes* (Gaisser *et al.*, 1997); lane 13, *S. platensis* (Furumai and Suzuki, 1975); lane 14, *S. griseoflavus* (Feng, 1995); lane 15, *S. vioreceoruber* Tü22 (Bechthold *et al.*, 1995); lane 16, *S. griseus* (Pissowotzki *et al.*, 1991; Distler *et al.*, 1992). C. Lane 17, *S. sp.* MCT846 (Lee *et al.*, 1996); lane 18, *S. niveus* (Hoggarth and Ritchie, 1995); lane 19, *S. nogalator* (Wiley *et al.*, 1977); lane 20, *S. venezuelae* (Facey *et al.*, 1996); lane 21, *S.* sp. SCC-2136 (Chu *et al.*, 1993); lane 22, *Actinomadura* sp. D. Lane 23, *Amycolatopsis orienthalis* subsp. *Orientalis* (Sheldrick *et al.*, 1978); lane 24, *Actinomudura roseorufa* (Funayama *et al.*, 1992); lane 25, *S. thermotolerans* (Arisawa *et al.*, 1995); lane 26, *S. fradiae* (Krügel *et al.*, 1996); lane 27, *S. amvofaciens* (Malet *et al.*, 1992).

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   99                                                    Homologue-2
1  SHVQRSILDA SVFVRTNVHG TQTLLDAATR HGVADRSSFV QVSTDEVYGS
2  .........G AQFVRTNVVG THTLIDAAHR AGVKTFVHI. ..STDEVYGS
3  SHVDRSIDGA SDFVRTNVTG TQTLLDRALR QGIETFVHI. ..STDEVYGS
4  .......... APFVTTNVLG TQLLLDAAAR HGVGRFLH.. .VSTDEVYGS
5  .......... ..FVRTNVAG TQTLLDACLA AGTERFLH.. .VSTDEVYGS

   149
1  LEEGAWTEDH PLLPNSPYSA SKASSTCSSA PVTAHHGLNV SITRCSNNYG
2  IDEGSWPETH PLEPNSPYSS AKASSDLIAL SYHRTHGLDV R
3  IDAGSWPDTA PVTPNSLYSA AKASSDLVAL A
4  IPVGSWSEES PLVPNSPYAA TKAGSELSAL ACHRTHGMDV.
5  YSRGRWTEDH PLLPNSPYAA SKASSTCSSA PV
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Fig. 5. The comparison of the deduced amino acid sequences of PCR products with the known dehydratase. Lane 1, StrE from *S. griseus*; Lane 2, PCR product from *S. peucetius* ATCC29050; Lane 3, *S. argillaceus*; Lane 4, *S. spetabilis*; and Lane 5, *S. achromogenes* var. *rubradiris* NRRL3061.

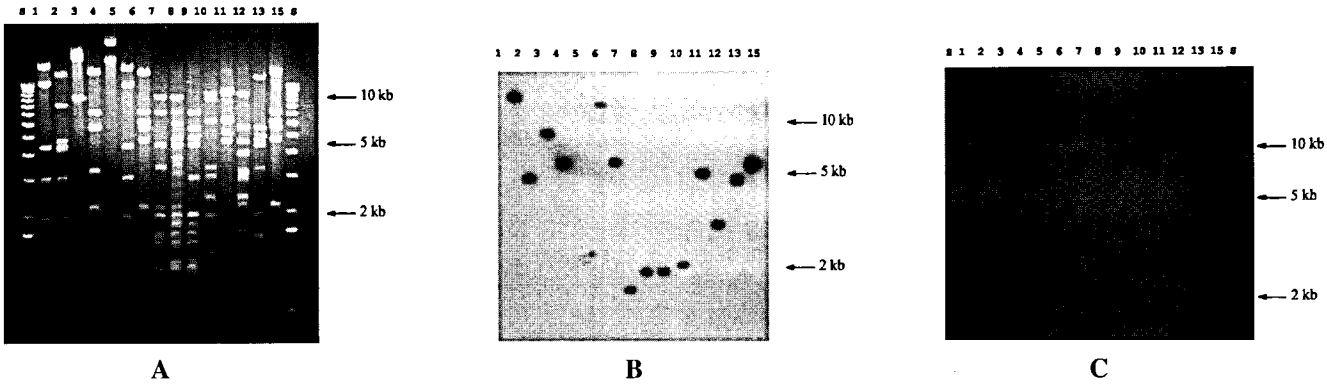

Fig. 6. A. Agarose gel electrophoresis. pLS15 was digested with several restriction enzymes; 1, *Bam*HI; 2, *Bam*HI and *Eco*RI; 3, *Eco*RI; 4, *Pst*I and *Eco*RI; 5, *Hind*III; 6, *Eco*RI and *Hind*III; 7, *Pst*I and *Hind*III; 8, *Bam*HI and *Pvu*II; 9, *Pvu*II; 10, *Pst*I and *Pvu*II; 11, *Bam*HI and *Sph*I; 12, *Sph*I; 13, *Pst*I and *Sph*I; 14, *Bam*HI and *Pst*I; 15, *Pst*I. B. Autoradiograph of hybridization with the ^{32}P-labeled PCR DNA. C. Autoradiograph of hybridization with the ^{32}P-labeled *actI*.

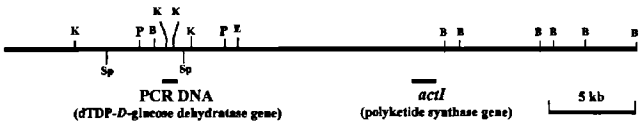

Fig. 7. Map of pLS15 cloned from *S. argillaceous*.

S. spectabilis produces an aminoglucoside antibiotic spectinomycin which consists of a deoxysugar and an aminoinositol (Price, 1986; Shaw *et al.*, 1993; Malpartida *et al.*, 1995). Eight cosmid clones (pSPM1 to 8) were screened from the genomic cosmid library of *S. spectabilis*. Partial DNA sequence of pSPM8 revealed genes homologous to a dTDP-D-glucose 4,6-dehydratase gene, a glucose-1-phosphate thymidylyltransferase gene, a resistance gene, an aminotransferase gene, and a glycosyltransferase gene (Sohng, unpublished data).

Streptomyces sp. MCT846 produces a macrolide antibiotic elaiophylin which is a 16-membered unsaturated bis-lactone with *L*-oliose (2-deoxyfucopyranose) (Lee *et al.*, 1996). Eight cosmid clones were screened from the genomic cosmid library of *Streptomyces* sp. MCT846. The cosmid clones were digested with a restriction enzyme and blotted to the *eryA* gene probe. Several DNA fragments were bound strongly to the gene probe, which is the typical result for the type II polyketide synthase gene. Total DNA fragments of about 30 kb were bound to the gene probe. This DNA size is well consistent with that of the anticipated biosynthetic gene cluster for the 16-membered lactone elaiophylin (Sohng, unpublished data).

We have isolated a part of the dTDP-D-glucose 4,6-dehydratase gene from 27 actinomycetes by PCR and used it as a gene probe to clone the biosynthetic gene clusters of secondary metabolites containing deoxysugars. Four gene clusters were cloned from *S. argillaceus*, *S. achromogenes*, *S. spectibilus*, and *Streptomyces* sp. MCT846 and the genes flanking the isolated dehydratase gene were analyzed by Southern blot and partial DNA sequencing. The results demonstrated that *eryA*, AHBA synthase gene, or *actI* gene homologues reside in the cloned gene clusters. In conclusion, we successfully cloned the biosynthetic gene clusters for the antibiotics mithramycin (aromatic polyketide), rubradirin (ansamycin antibiotic), elaiophylin (macrolide polyketide), and spectinomycin (aminoglucoside antibiotic). The PCR product of the dehydratase gene, therefore, can be used as a universal gene probe to isolate the biosynthetic gene clusters of various deoxyglycosylated secondary metabolites produced from actinomycetes. The biosynthetic pathways of the identified gene clusters are being studied and applied to create novel secondary metabolites by gene manipulation.

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