J. Microbiol. Biotechnol. (2007), **17**(12), 1909–1921

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Discovery and Molecular Engineering of Sugar-containing Natural Product Biosynthetic Pathways in Actinomycetes

OH, TAE-JIN[1], SANG JOON MO[2], YEO JOON YOON[2], AND JAE KYUNG SOHNG[1]*

[1]*Institute of Biomolecule Reconstruction (IBR), Department of Pharmaceutical Engineering, SunMoon University, Asan 336-708, Korea*
[2]*Division of Nano Sciences and Department of Chemistry, Ewha Womans University, Seoul 120-750, Korea*

Received: September 5, 2007 Accepted: October 2, 2007

Abstract Significant progress has recently been made concerning the engineering of deoxysugar biosynthesis. The biosynthetic gene clusters of several deoxysugars from various polyketides and aminoglycosides-producing microorganisms have been cloned and studied. This review introduces the biosynthetic pathways of several deoxysugars and the generation of novel hybrid macrolide antibiotics *via* the coexpression of deoxysugar biosynthetic gene cassettes and the substrate-flexible glycosyltransferases in a host organism as well as the production of TDP-deoxysugar derivatives *via* one-pot enzymatic reactions with the identified enzymes. These recent developments in the engineering of deoxysugars biosynthesis may pave the way to create novel secondary metabolites with potential biological activities.

Keywords: Actinomycetes, combinatorial biosynthesis, deoxysugar, glycosyltransferase, natural product

Nature produces an amazing number of products that have useful biological activities with clinical applications, including antibiotics, and antitumor and immunosuppressive agents [10]. Natural products remain a consistent source of drug leads, with more than 49% of the new chemical entities reported being from microbial secondary metabolites between 1981 and 2002 [2, 52]. These bioactive secondary metabolites are the end products of complex multistep biosynthetic pathways, and approximately 75% of them are produced by members of the actinomycetes primarily belonging to the genus *Streptomyces*. There has been a great expansion of our knowledge in the biosynthetic pathways from which microorganisms produce bioactive secondary metabolites during the past decade. This has allowed researchers to explore the possibility of generating novel natural product derivatives with potentially greater or altered biological activities.

*Corresponding author
Phone: 82-41-530-2246; Fax: 82-41-544-2919;
E-mail: sohng@sunmoon.ac.kr

Recently, much attention has been focused on the ability of polyketide synthases (PKSs), which can be classified into types I, II, or III enzymes according to their structural and catalytic domain organizations, to synthesize complex chemical scaffolds. These giant multifunctional enzyme systems assemble a number of secondary metabolites mostly from simple building blocks such as carboxylic acids. The structural diversity of PKS products is further increased by attaching of deoxygenated sugars at different positions. In contrast to the complexity and diversity of PKSs, the enzymatic mechanisms leading to the formation of deoxygenated sugars are quite conserved. These deoxysugar components have been recognized as an important class of carbohydrates and have enjoyed increasingly widespread appreciation as essential biological molecules exhibiting a great range of activities including functions as a cellular adhesive, in the immune response, fertilization, as well as in the molecular recognition and affinity of its cellular target [55]. These sugars are transferred to the aglycone by glycosyltransferase (GT). Many efforts on creating novel microbial natural products have been aimed at finding approaches to incorporate different sugars into an aglycone or to glycosylate a different position of the aglycone. Other well-known sugar-containing microbial natural products are aminoglycoside antibiotics. They are among the first antibiotics to be used in a clinical setting, and are an important class of antibiotics against Gram-negative bacteria such as *Mycobacterium tuberculosis* and methicillin-resistant *Staphylococcus aureus* (MRSA) [9, 71], and also show potentially useful activity against human immunodeficiency virus (HIV) [46, 69].

Recently, various polyketide biosynthetic gene clusters have been reported, which include partially deoxygenated sugar components such as 6-deoxysugar, dideoxysugar, aminodeoxysugar, nitrodeoxysugar, and trideoxysugar [4, 48]. The key biosynthetic step in deoxysugar biosynthesis is the conversion of glucose-1-phosphate to TDP-4-keto-6-deoxy-D-glucose (TKDG) *via* TDP-D-glucose. When the genes encoding NDP-glucose synthase and NDP-D-glucose

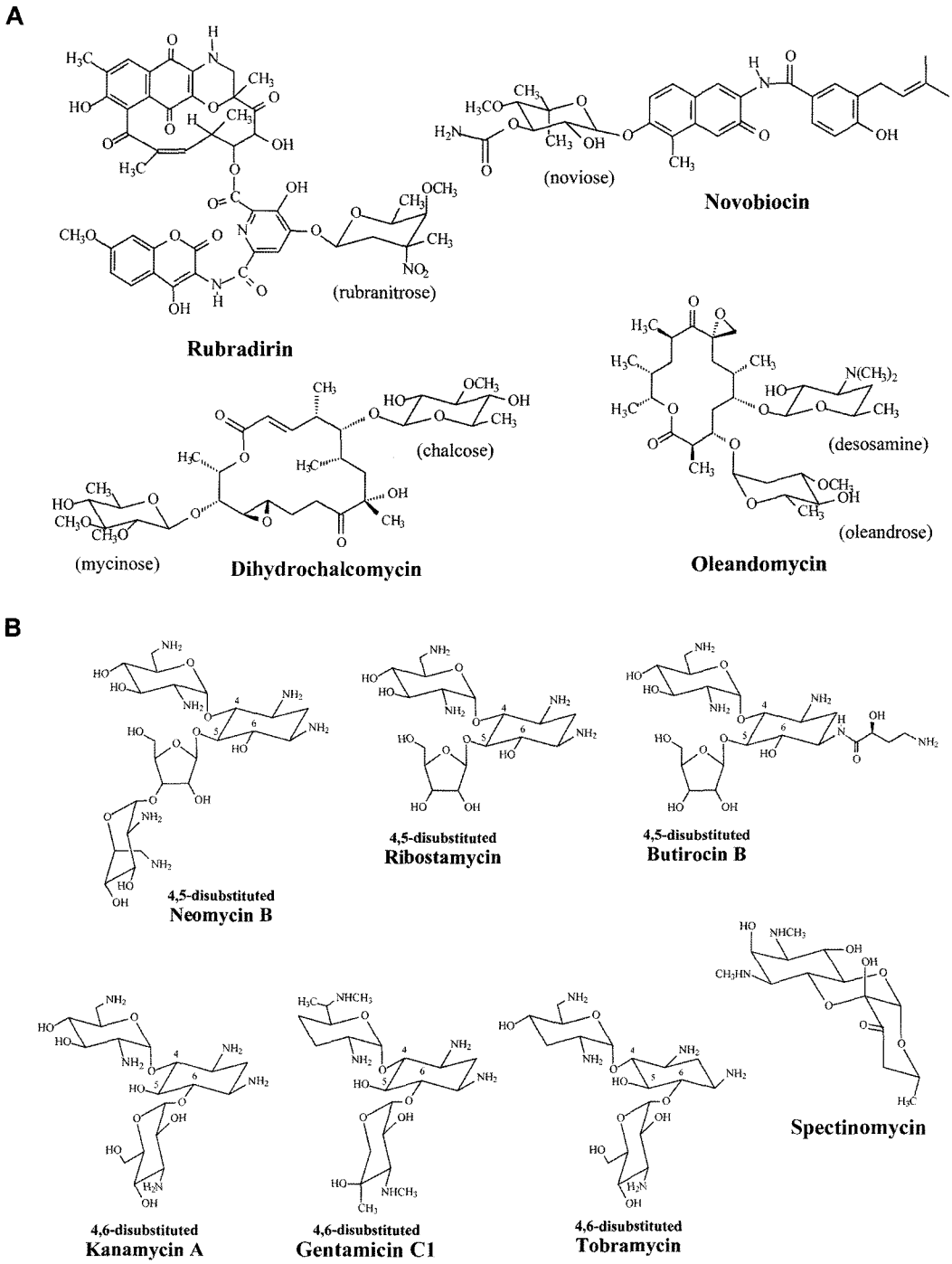

Fig. 1. The chemical structures of polyketides (**A**) and aminoglycosides (**B**) including several kinds of deoxysugars.

4,6-dehydratase are available, the gene clusters flanking these genes could be cloned to identify the entire biosynthetic gene cluster involved in the secondary metabolites associated with deoxysugars [59, 61]. The gene probes related to the deoxysugar (TDP-D-glucose synthase or/and TDP-D-glucose 4,6-dehydratase) were used to clone several secondary metabolite gene clusters [60] such as rubradirin (rubranitrose), novobiocin (noviose), dihydrochalcomycin (chalcose and mycinose), oleandomycin (desosamine and oleandrose), and pradimicin (Fig. 1A) [11, 24, 33, 61, 63]. Moreover, the biosynthetic studies of aminoglycosides have been carried out for compounds containing different sugars: spectinomycin (spectinose), neomycin (neosamines and D-ribose), butirosin (neosamines and D-ribose), ribostamycin

(neosamine C and D-ribose), gentamicin (garosamine and purpurosamine), kanamycin (kanosamine and neosamine C), and tobramycin (kanosamine and neosamine C) (Fig. 1B) [22, 28–31, 36, 59, 64].

It is now possible to introduce biosynthetic engineering into a natural product by rational manipulation of the gene cluster governing its biosynthesis. To identify and obtain several hypothetical products and to use the enormous genetic potential in an efficient way, combinatorial biosynthesis and heterologous gene expression approaches are required. The strategies developed thus far that are generally employed to glycosylate natural products include total synthesis or semisynthesis [12, 76, 78], glycorandomization [13, 39], and *in vivo* pathway engineering (referred to as combinatorial biosynthesis) [32, 40, 57], which relies upon the coexpression of sugar biosynthetic gene cassettes and GT using an endogenous or exogenously delivered aglycone. These new technologies helped us to manipulate the genes of natural product biosynthetic pathways in order to generate a hybrid compound that may exhibit novel properties and efficacies against problematic and resistant pathogens. This review, therefore, attempts to highlight the general aspects of the heterologous production of the novel derivatives of previously mentioned polyketides and aminoglycosides, as well as enhanced production of desired natural products using *S*-adenosyl-L-methionine (SAM) synthetase and regulatory genes, which were thus shown to be functional in the closely related host organisms.

RECENT EXAMPLES OF THE BIOSYNTHETIC GENE CLUSTER OF NATURAL PRODUCTS FROM ACTINOMYCETES

Rubradirin belongs to the family of antibiotics known as ansamycins [74]. Rubradirin from *Streptomyces achromogenes* var. *rubradiris* NRRL 3061 is comprised of four distinct moieties; rubransarol, 3-amino-4-hydroxy-7-methoxycoumarin (AMC), 3,4-dihydroxydipicolinate (DHDP), and an unusual nitrosugar, 2,3,6-trideoxy-3-*C*-4-*O*-dimethyl-3-*C*-nitro-D-*xylo*-hexose (D-rubranitrose) (Fig. 1A). The putative functions of a total of 23 genes were determined *via* sequence comparison [61]. Eight genes, *rubK*, *L*, *M*, *N*, *G*, *H*, *J*, and *L*, were implicated in the biosynthesis of the common ansamycin precursor; two PKS genes, *rubA* and *B*, which consisted of 6 modules, were involved in rubransarol biosynthesis; eight genes, *rubN1* to *N8*, were putatively related to TDP-D-rubranitrose biosynthesis [38, 49]; and five genes, *rubC1* to *C5*, were involved in AMC and DHDP biosynthesis. It was demonstrated that the nitro group in D-rubranitrose is an air-oxidation product of the nitroso analog (**9**), and it has been predicted that the nitrosugars in those antibiotics are also air-oxidation products by analogy [3]. As shown in Fig. 2A, the D-rubranitrose biosynthetic pathway might require eight steps, which would include TDP-D-glucose synthase (RubN1), 4,6-dehydratase (RubN2), 2,3-dehydratase (RubN3), 3-aminotransferase (RubN4), 3-*C*-methyltransferase (RubN5), 4-ketoreductase (RubN6), *O*-methyltransferase (RubN7), and amine oxidase (RubN8). The conversion of TKDG (**1**) to TDP-2,3,6-trideoxy-3-amino-3-methyl-D-glucose (**8**) by the consecutive catalysis of 2,3-dehydratase, 3-aminotransferase, and 3-*C*-methyltransferase was reported in the biosynthesis of L-epivancosamine, which is a component of chloroeremomycin, one of the vancomycin family glycopeptide antibiotics [6].

Heide and coworkers isolated a novobiocin biosynthetic gene cluster from *Streptomyces spheroides* NCIB 11891, including the presence of 23 open reading frames (ORFs) with a possible role in noviose biosynthesis [63]. Noviose attaches to the 7' position of the coumarin core *via* a glycosidic linkage (Fig. 1A), and it represents an essential component that confers the biological activity of this compound [21]. Five ORFs (*novV*, *novT*, *novW*, *novU*, and *novS*) have been predicted as the noviose biosynthetic genes. The *novW*, *novU*, and *novS* gene products represent TDP-4-keto-6-deoxy-D-glucose 3,5-epimerase (NovW), *C*-methyltransferase (NovU), and TDP-D-glucose-4-ketoreductase (NovS). Several reactions were carried out, alone or coupled, involving these three enzymes, NovS, NovU, and NovW, in order to envisage their possible biosynthetic roles in noviose formation. The formation of **3** only from the reaction mixture containing TKDG, NovU, and NovW out of a number of various mixtures indicates that NovU acts only on the reaction product **2** from NovW catalysis to give **3**, as shown in Fig. 2A. Compound **3** probably undergoes reduction by NovS to generate TDP-D-noviose. The accumulated formation of TDP-L-rhamnose in the mixture indicates that **2** is reduced by NovS at a much higher rate than the methylation by NovU, thus suggesting the substrate flexibility of NovS [27]. These results are in agreement with the previous reports that some of the 4-ketoreductases in deoxysugar biosynthetic pathways are flexible to their substrates and reduce the *C*-4-carbonyl carbon of both methylated and unmethylated sugar intermediates [57]. Compound **2** is needed as a precursor in the biosynthesis of TDP-L-rhamnose [14]. The formation of TDP-L-rhamnose in the coupled enzymes of NovW and NovS clearly indicated that NovW acts as a TDP-4-keto-6-deoxy-D-glucose 3,5-epimerase in the noviose biosynthetic pathway [67] (Fig. 2A).

Dihydrochalcomycin, a 16-membered macrolide antibiotic produced by *Streptomyces* sp. KCTC 0041BP, contains chalcose and mycinose (Fig. 1A), and it also exhibits antimicrobial activity against Gram-positive bacteria. The genes required for the synthesis of these two deoxysugar moieties, D-chalcose and D-mycinose, were found in the cluster. TKDG is produced from glucose-1-phosphate through the action of the gene products of *gerE* and

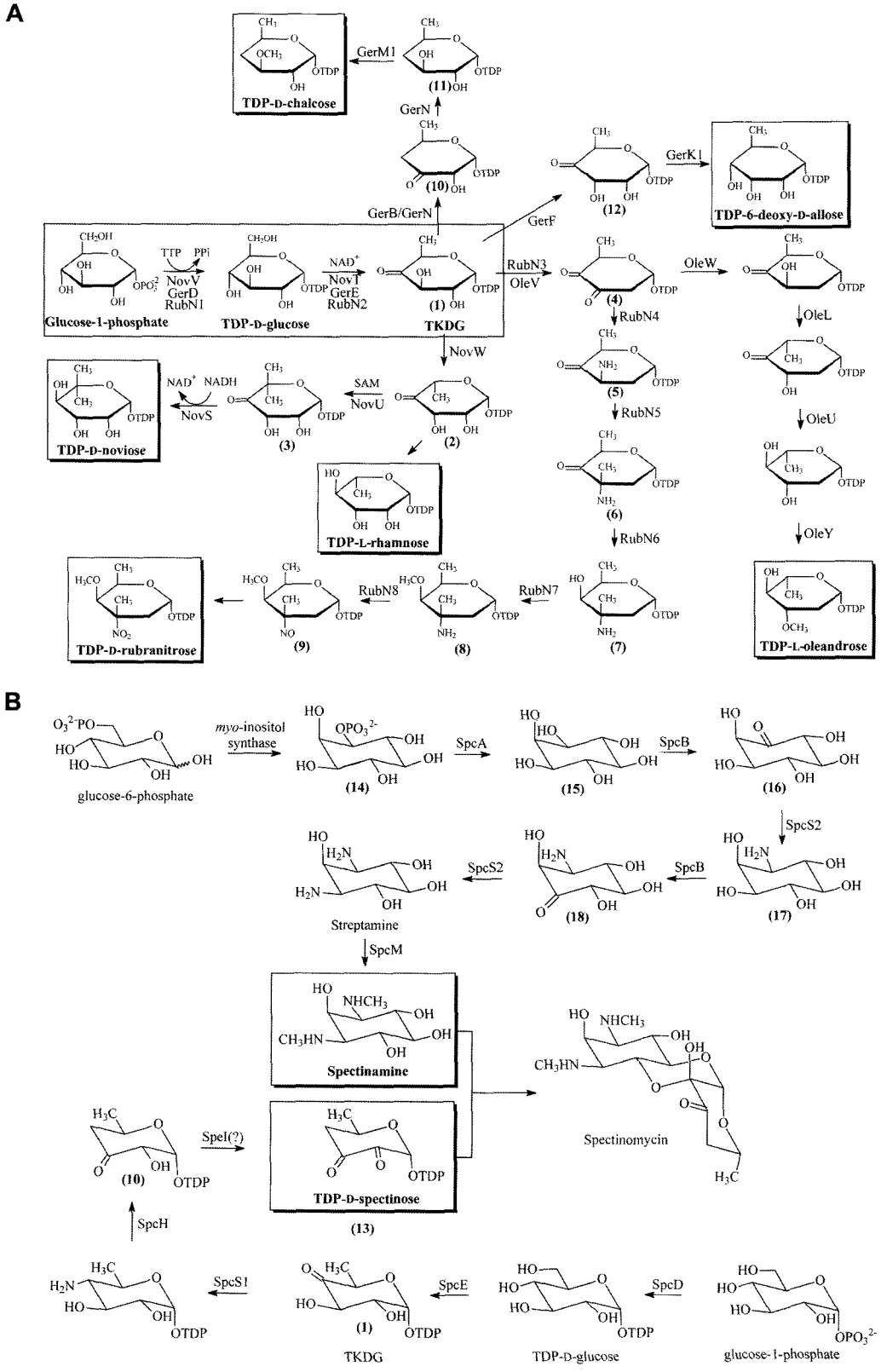

Fig. 2. The proposed biosynthetic pathway of deoxysugar derivatives from various natural products.
A. Rubradirin, novobiocin, dihydrochalcomycin, oleandomycin, and spectinomycin. **B**. Streptamine-containing aminoglycoside-spectinomycin. **C**. 2-DOS-containing 4,5-disubstituted aminoglycoside-neomycin, butirosin, and ribostamycin, and 2-DOS-containing 4,6-disubstituted aminoglycoside-gentamicin, kanamycin, and tobramycin.

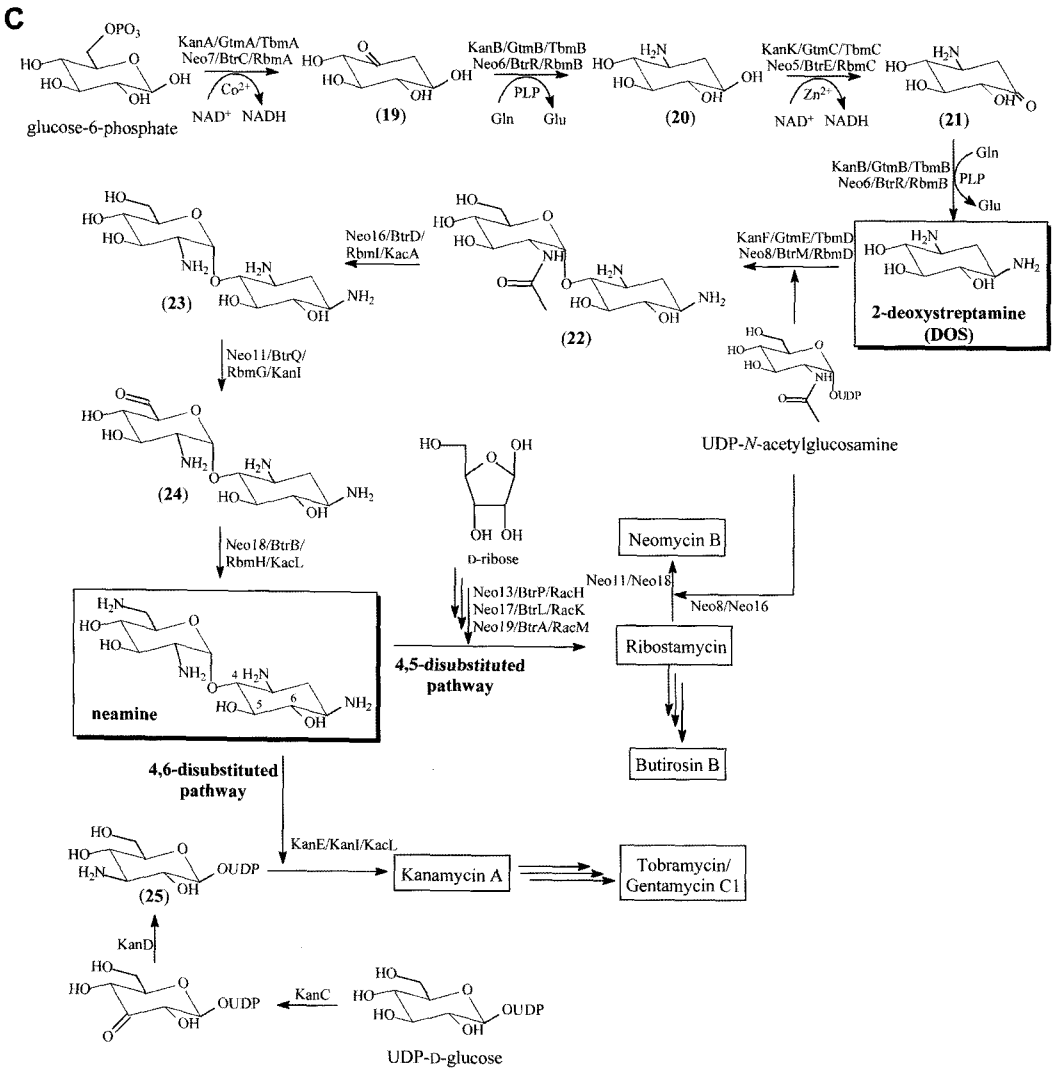

Fig. 2. Continued.

gerD [41, 42], which is very similar to SchS6, a probable glucose-1-phosphate thymidylyltransferase involved in the conversion of dTTP and glucose-1-phosphate to TDP-D-glucose and pyrophosphate from *Streptomyces* sp. SCC2136 [15]. As shown in Fig. 2A, the synthesis of TDP-D-chalcose involves deoxygenation at *C*-4, which requires the reduction of the radical formed after the removal of the oxygen atom. GerB and GerN, which are homologs of the desosamine enzymes DesI and DesII, respectively, carry out these reactions in a similar way to the pikromycin pathway to produce the *C*-4 deoxygenated sugar (**10**) [8, 75]. The next step after deoxygenation is the reduction of the 3-keto group of the pathway intermediate (**11**). The 3-*O*-methylation of TDP-4,6-dideoxy-D-glucose to produce TDP-D-chalcose is carried out by GerM1, which is very similar to SpnH, a probable methyltransferase involved in the *O*-methylation of the rhamnose precursor [72]. The synthesis of mycinose requires two enzymes, a 3-epimerase and a 4-ketoreductase, which are encoded by GerF and GerK1 [58, 68]. The conversion of the 6-deoxy-D-allose residue to D-mycinose after attachment to the macrolactone requires the action of two *O*-methyltransferases that are encoded by GerM2 and GerM3, each *ca.* 73% identical to their respective counterparts in the tylosin biosynthetic cluster, TylE and TylF. They are also proposed to act after the sugar has been attached to the backbone.

In general, aminoglycosides are classified into two major groups, the streptamine-containing aminoglycosides and 2-deoxystreptamine (2-DOS)-containing aminoglycosides, depending upon their structural components. Spectinomycin is the streptamine-containing aminoglycoside antibiotic produced by *Streptomyces spectabilis* [50]. This antibiotic has a unique tricyclic structure in which a single sugar component, TDP-D-spectinose, is linked to the diaminocyclitol moiety (spectinamine) by β-glycosidic and hemiketal bonds (Fig. 1B) [17]. The gene cluster related to spectinomycin

was cloned from *S. spectabilis* [59]. Six steps were proposed for the biosynthetic pathway to TDP-D-spectinose. TDP-D-glucose synthase (*spcD*) and 4,6-dehydratase (*spcE*) were involved in the biosynthesis of spectinose (**13**). The *C*-4 deoxygenation of spectinose can be proposed to be the same as the *C*-4 deoxygenation in the desosamine and chalcose biosynthetic pathways. These reactions are carried out by SpcS1 and SpeH, which are homologs of the desosamine enzymes DesI and DesII, or the chalcose enzymes GerF and GerK1 [58, 68], respectively. The oxidation of the 2-keto group by SpeI (dehydrogenase) and the hydration of the 3-keto group by SpcF (dehydratase) are expected to follow the deoxygenation (Fig. 2B). For the biosynthesis of spectinamine, there are four related genes including *speA*, *speB*, *spcS2*, and *spcM*. The *speA* gene, which functions as a *myo*-inositol-1-monophosphatase, is a key enzyme required for the conversion of *myo*-inositol (**14**) to *myo*-inositol-1-monophosphate (**15**). The *speB* gene is 73% identical to the *myo*-inositol dehydrogenase of *Streptomyces flavosporius*, which is also a key enzyme required for the conversion to *scyllo*-inosose (**16**) [25]. The *spcS2* gene, which encodes L-glutamine: *scyllo*-inosose aminotransferase, catalyzes a transamination reaction at the carbonyl carbon of *scyllo*-inosose to form *scyllo*-inosamine (**17**) in the streptamine biosynthetic pathway [64, 65]. Finally, the *spcM* gene, which encodes the *N*-methyltransferase involved in the conversion of streptamine to spectinamine, is 31% identical to the 3-*N*-methyltransferase of *Streptomyces hygroscopicus*, a hygromycin B producer.

Structurally, the majority of the aminoglycosides share a common aglycon, 2-DOS, which is linked with other sugar subunits *via* *O*-glycosidic linkages. Neomycin (Neo) and butirosin (Btr) are close structural relatives that both contain the ribostamycin (Rbm) substructure, and they belong to the same class of 2-DOS-containing 4,5-disubstituted aminoglycosides that share two common subunits including neosamine and D-ribose. Moreover, a second class of 2-DOS-containing 4,6-disubstituted aminoglycosides includes kanamycin (Kan), tobramycin (Tbm), and gentamicin (Gtm). The biosynthesis of these antibiotics had been studied a few decades ago, and the studies of biosynthetic pathway addressing the formation of these antibiotics have recently been proposed with the isolation of all biosynthetic gene cluster [22, 28–31, 36, 59, 64]. As shown in Fig. 2C, the biosynthesis of 2-DOS from glucose-6-phosphate has been extensively characterized by expressing each gene that encodes 2-deoxy-*scyllo*-inosose synthase, L-glutamine: 2-deoxy-*scyllo*-inosose aminotransferase, and dehydrogenase responsible for the products 2-deoxy-*scyllo*-inosose (**19**), 2-deoxy-*scyllo*-inosamine (**20**), and 3-amino-2,3-dideoxy-*scyllo*-inosose (**21**), respectively (Fig. 2C) [23, 37, 66]. The 2-DOS is proposed to be converted to neamine by the addition of the primary metabolite UDP-*N*-acetylglucosamine at *C*-4 to give 2'-*N*-acetylparomamine (**22**), which is deacetylated by a *N*-acetyltransferase, BtrD [70]. Paromamine (**23**) is converted to 6'-dehydro-6'oxoparomamine (**24**) and neamine through sequential dehydrogenation and pyridoxal phosphate (PLP)-dependent transamination [16] at *C*-6' by hitherto-unidentified enzymes (Fig. 2C). The remainder of the pathway leading from neamine to the 4,5-disubstituted aminoglycosides (ribostamycin, butirosin, and neomycin) and the 4,6-disubstituted aminoglycosides (kanamycin, tobramycin, and gentamicin) still awaits experimental elucidation.

COMBINATORIAL BIOSYNTHETIC STRAGEGIES USING THE HETEROLOGOUS EXPRESSION

Structural Diversification of Polyketide by Substrate-flexible GT and Its Auxiliary Protein

More than 50 genes encoding GT are being reported from the gene clusters of antibiotic-producing Actinomycetes. The process of generating glycosylated derivatives *via* combinatorial biosynthesis relies upon the access to an array of flexible GTs. It was recently discovered that the macrolide GT DesVII required an auxiliary protein, DesVIII, for its *in vitro* and *in vivo* activities [5, 19, 20], as is the case of AknS/AknT (aclacinomycin) [47], TylM2/TylM3 (tylosin) [51], and MycB/MydC (mycinamycin) [51]. In these DesVII/DesVIII systems, when the native auxiliary protein was replaced with a heterologous activator protein, the efficiency of glycosylation was affected. They have been shown to be especially "flexible" in accepting different deoxysugars, which leads to further structural variations [18, 43]. These unique features in generating structural variability can be applied to the design of strategies that can be used to accomplish the combinatorial biosynthesis of novel hybrid macrolides.

In Vivo Pathway Engineering: New Deoxysugar-glycosylated Derivatives of Methymycin/Pikromycin

Solenberg *et al.* [62] were the first to describe the use of heterologous GT genes for the production of hybrid glycopeptide antibiotics. This landmark experiment takes advantage of the existence of an intracellular pool of nucleotide-activated sugars in microorganisms by several approaches that are shown in Fig. 3.

The gene clusters of the deoxysugar could be used to construct the cassettes containing the genes that code for the two enzymes, TDP-D-glucose synthase (*desIII*) and TKDG (*desIV*), which catalyze the two common steps in the biosynthesis of a key intermediate of the deoxysugar. This plasmid was integrated into the genomic DNA of *Streptomyces venezuelae*, engineered by the deletion of the entire gene cluster related to the biosynthesis of the endogenous deoxysugar (TDP-D-desosamine) in order to obtain a mutant strain [18]. This strain could be used as a

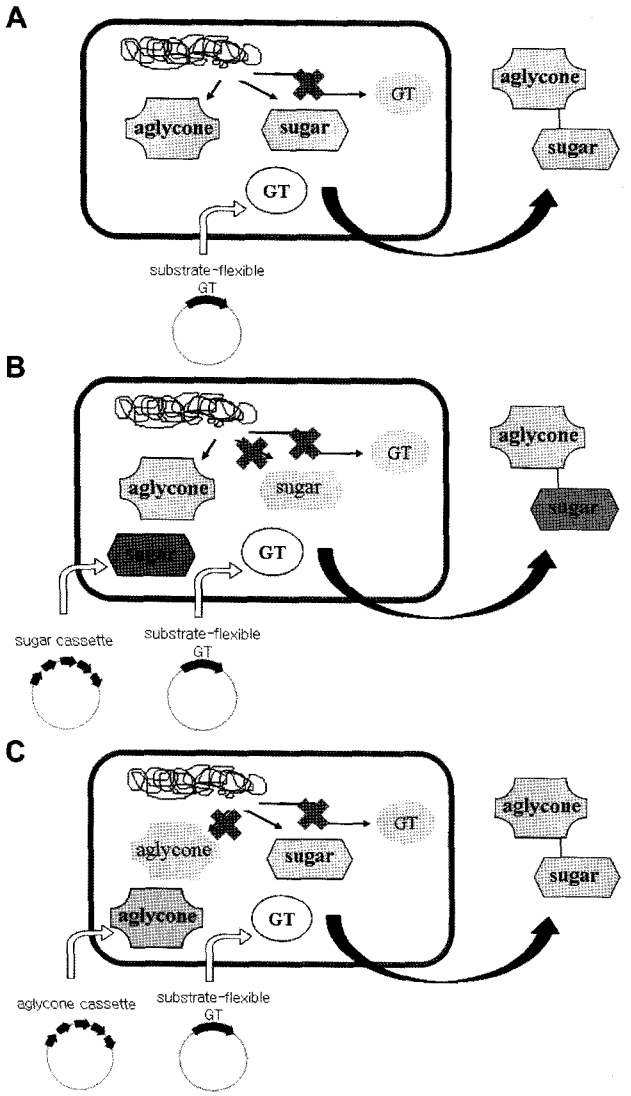

Fig. 3. A concept for the generation of novel glycosylated natural products by combinatorial biosynthesis and genetically engineered biotransformation.
A. Formation of derivative in a GT-deficient mutant by expression of the flexible GT. **B.** Formation of derivative in a sugar- and GT-deficient mutant by expression of the other sugar cassette genes and flexible GT, respectively. **C.** Formation of derivative in an aglycone- and GT-deficient mutant by expression of the flexible GT. The aglycone is endogenously synthesized (**A** and **B**) or exogenously supplied by feeding (**C**).

starting point for the biosynthesis of various deoxysugars. Another plasmid was constructed to contain the gene encoding a GT for the attachment of the intermediate sugar to the aglycone and four more genes (*desVIII*, *oleV*, *oleW*, and *urdR*) that are involved in the biosynthesis of TDP-D-olivose from TKDG. This plasmid was transformed to the mutated *S. venezuelae* to produce olivosyl-methymycin and olivosyl-pikromycin [18] (Fig. 4A). In addition, the heterologous sugar genes encoding 3,5-epimerase (*orf9*) and TDP-4-keto-6-deoxy-glucose 4-aminotransferase (*gerB*) [24] were also introduced into the same mutant in order to accomplish the biosynthesis of novel derivatives that contained the deoxyaminosugar TDP-4-amino-4,6-dideoxy-L-glucose [unpublished]. Our results demonstrate that this mutant generates new hybrid macrolide derivatives, including TDP-4-amino-4,6-dideoxy-L-glycosylated YC-17, methymycin, novamethymycin, and pikromycin, from a 12-membered ring aglycone (10-deoxymethynolide) and a 14-membered ring aglycone (narbonolide) (Fig. 4B). These results demonstrate a successful attempt to engineer the deoxysugar pathway to generate novel hybrid secondary metabolites.

In Vitro Pathway Engineering: Syntheses of TDP-Deoxysugar Derivatives *via* a One-Pot Enzymatic Reaction

TKDG is a key intermediate for the other TDP-D- and TDP-L-deoxysugar derivatives. A typical synthetic method for TKDG is using a two-enzyme system; *i.e.*, TDP-D-glucose synthase and TDP-D-glucose 4,6-dehydratase with glucose-1-phosphate and TTP as substrates. The high cost of the TTP required for this process limits the large-scale production of TKDG. We devised an economical enzymatic production method of TKDG starting from TMP, which is a much cheaper substrate than TTP. The enzymatic production methods for TKDG were developed to start from TMP, acetyl phosphate, and glucose-1-phosphate [53]. The four enzymes required for this process are TMP kinase, acetate kinase, TDP-D-glucose synthase, and TDP-D-glucose 4,6-dehydratase (Fig. 5). In this reaction, TMP-kinase converts TMP to TDP, and acetate kinase regenerates the ATP and converts TDP to TTP. TDP-D-glucose synthase is responsible for the conversion of TTP and glucose-1-phosphate to TDP-D-glucose, and finally TDP-D-glucose 4,6-dehydratase converts TDP-D-glucose to TKDG. TKDG could be conveniently synthesized using the enzymatic extracts in a one-pot batch system with four enzymes that are overexpressed using the T7 promoter system in *E. coli* BL21. A TKDG conversion yield of approximately 95% was obtained based on an initial TMP concentration of 20 mM, 20 mM $MgCl_2$, 60 mM acetyl phosphate, 80 mM glucose-1-phosphate, and 1 mM ATP [53].

TDP-L-rhamnose was also prepared on a large scale from TKDG by executing a reaction involving two enzymes. TDP-4-keto-6-deoxy-D-glucose 3,5-epimerase (OleL) and TDP-4-keto-hexose reductase (OleU), which are responsible for the conversion of TKDG to TDP-L-rhamnose, were obtained from *Streptomyces antibioticus* Tü99, an oleandomycin producer, and were functionally expressed in *E. coli* using the pET and pRSET expression systems, respectively [1]. The two enzymes were combined with an enzymatic process for the regeneration of NADH using gluconate dehydrogenase (GDH) and glucose. A TDP-L-rhamnose conversion yield of approximately 91% was

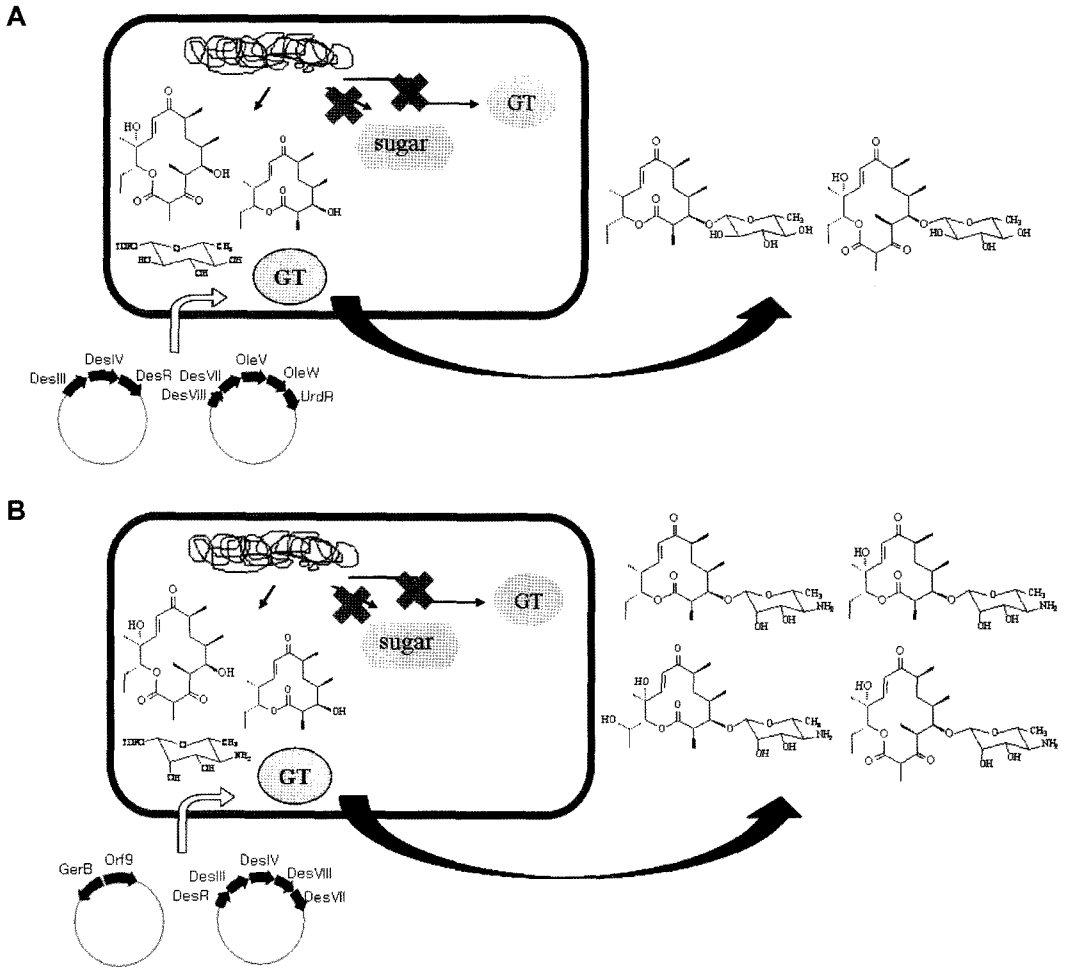

Fig. 4. Formation of novel glycosylated natural products in a sugar biosynthetic gene cluster-deficient mutant of *Streptomyces venezuelae* by expression of the sugar gene cassette.
A. Olivosyl-pikromycin and olivosyl-methymycin. **B.** TDP-4-amino-4,6-dideoxy-L-glycosylated YC-17, methymycin, novamethymycin, and pikromycin.

obtained based on initial concentrations of 20 mM TKDG and 10 mM NADH (Fig. 6) [27]. In addition, TDP-L-rhamnose can be prepared from TMP and glucose-1-phosphate in a one-pot reaction that includes six enzymes. Three of the six enzymes, TMP kinase, acetate kinase, and TDP-D-glucose synthase, were related to the enzymatic process of TKDG biosynthesis. This system allowed us to achieve the preparative scale synthesis of TDP-L-rhamnose using TMP and glucose-1-phosphate as starting materials.

Various deoxysugars, such as TDP-6-deoxy-D-allose, TDP-D-frucosamine, and TDP-4-amino-4,6-dideoxy-D-glucose, were prepared from TKDG following the synthesis of TDP-L-rhamnose (Fig. 6). TDP-6-deoxy-D-allose was synthesized with 3,5-epimerase (GerF) and TDP-4-ketoreductase (GerK1) using an enzymatic process for the regeneration of NADH [58, 68]. TDP-D-frucosamine and TDP-4-amino-4,6-dideoxy-D-glucose were also prepared by aminotransferases (WecE and GerN). Their amine source is a glutamic acid, and a PLP is involved as a cofactor.

Approaches for the Enhanced Productivity of Secondary Metabolites

Although many of the biochemical studies of natural product biosynthetic enzymes have been extremely successful in the generation of novel natural product derivatives and unusual chemical conversions, our ability to rationally redesign biosynthetic pathways using combinatorial biosynthesis is often limited by our lack of understanding of the interplay between the many enzymes in the organisms. Despite this, recent approaches should allow an improvement in productivity of secondary metabolites with some tailoring enzymes such as the substrate-flexible GTs [26] and cytochrome P450 [44, 54, 73]. Some genes involved in the regulation of secondary metabolism, such as SAM synthetase [7, 35, 45, 77] and the global antibiotics-stimulating regulatory gene [34, 56], are also useful for the productivity improvement.

We noted above that the biosynthetic gene clusters for glycosylated polyketides and aminoglycosides that have

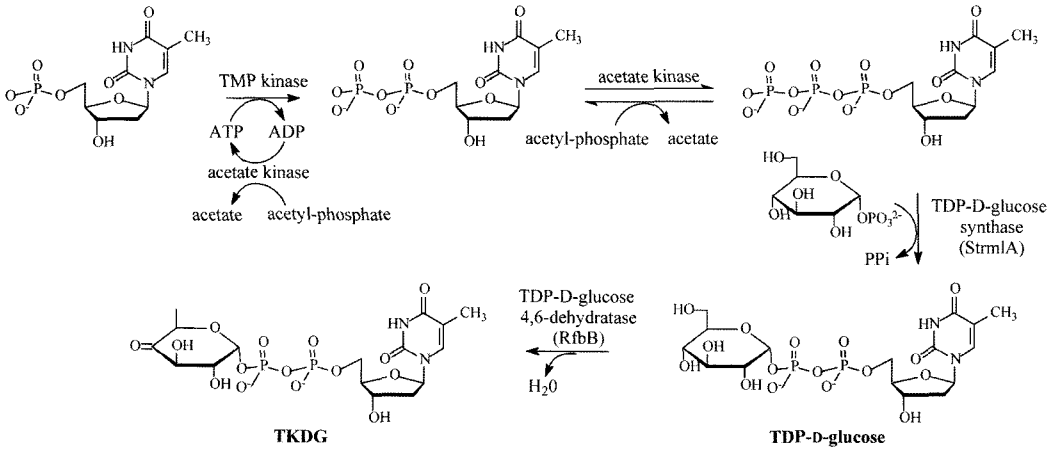

Fig. 5. Schematic diagram of the production system for TKDG from glucose-1-phosphate and TMP.

been sequenced contain the particular deoxysugars as activated NDP deoxysugars. The genes encoding the GT are also generally found in the same gene clusters. The promiscuity of DesVII/DesVIII to the pikromycin aglycone clearly showed higher production of new hybrid macrolide derivatives including 12-, 14-, and 16-membered ring aglycones by heterologous expression (Fig. 4) [26] than other GTs. The cytochrome P450 monooxygenase PikC, from the pikromycin producer *S. venezuelae*, was also observed to hydroxylate the unnatural substrate indole to indigo. From enzyme kinetic studies, the mutant enzyme F171Q of PikC showed an approximately five-fold higher catalytic efficiency. Therefore, these results demonstrate the promising application of P450 to generate a range of novel natural products with enhanced productivity [44].

It is known that overexpression of SAM synthetase or exogenous addition of SAM enhances the production of secondary metabolites, actinorhodin and oleandomycin from *Streptomyces coelicolor* [35] and *Streptomyces antibioticus* [77], respectively. In order to discover a novel compound as a signal molecule to produce actinorhodin instead of SAM, several compounds were synthesized and tested, and their actinorhodin production was analyzed. Of these, the molecules containing both bulky substituents at the *C*-6 position of adenine and the long 5'-alkyl chain of adenosine showed better productivities of actinorhodin than SAM [7, 45]. The 63 amino-acid-encoding *afsR2* is a relatively new global antibiotics-stimulating regulatory gene identified from *Streptomyces lividans*. The site-directed mutagenesis in the conserved area among known

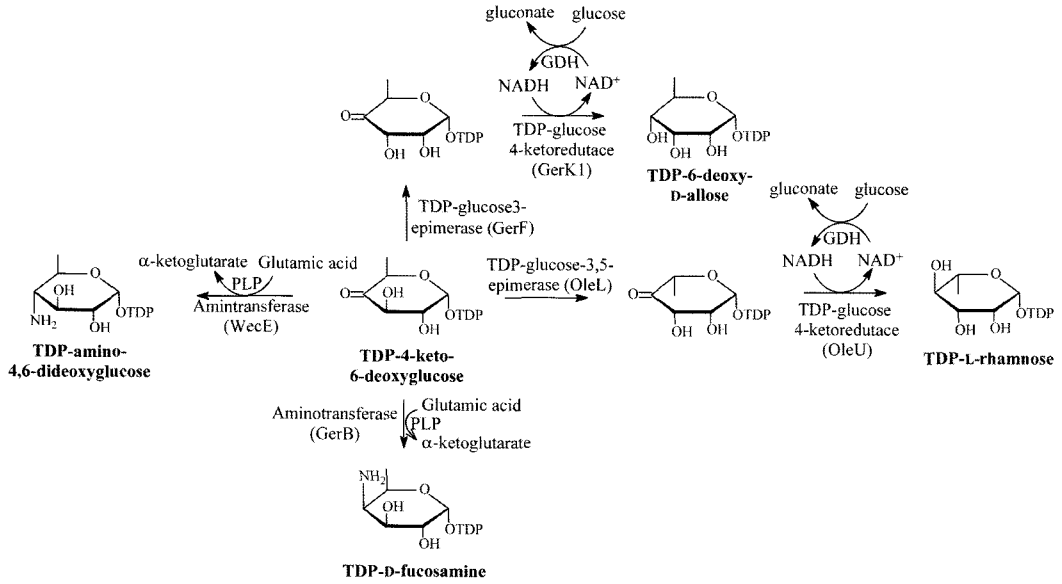

Fig. 6. The production scheme of TDP-deoxysugars from TKDG.

Sigma 70 family proteins changed the AfsR2's activity. These results showed that the C-terminal region of AfsR2 is functionally important for its antibiotics-stimulating capability [34].

CONCLUSION

These recent progresses opened the possibility of generating novel glycosylated compounds using *in vivo* and *in vitro* pathway engineering techniques. An efficient GT toolbox must be generated to attach unnatural deoxysugars to the diverse aglycones. All deoxysugar genes, including GT, may be attractive targets for use in the generation of novel natural products in the near future, and it will also contribute to the possible enzymatic synthesis of a wide variety of glycosylated derivatives. Thus, the programmed manipulation of the genes that encode the enzymes in the deoxysugar biosynthetic pathways shows great potential for use in the redesign of natural products to create new activities.

Acknowledgments

This work was supported by a grant (20050401-034-682-006-02-00) from the BioGreen 21 Program and a grant (Code#2005-005-J16002) from the Korea Research Foundation, Republic of Korea. This work was also supported by the Marine and Extreme Genome Research Center Program, Ministry of Maritime Affairs and Fisheries, Republic of Korea (to Y. J. Y).

REFERENCES

1. Aguirrezabalaga, I., C. Olano, N. Allende, L. Rodriguez, A. F. Brana, C. Mendez, and J. A. Salas. 2000. Identification and expression of genes involved in biosynthesis of L-oleandrose and its intermediate L-olivose in the oleandomycin producer *Streptomyces antibioticus*. *Antimicrob. Agents Chemother.* **44:** 1266–1275.
2. Baltz, R. H. 2005. Antibiotic discovery from Actinomycetes: Will a renaissance follow the decline and fall? *SIM News* **55:** 186–196.
3. Bannister, B. and B. A. Zapotocky. 1992. Protorubradirin, an antibiotic containing a *C*-nitroso-sugar fragment, is the true secondary metabolite produced by *Streptomyces achromogenes* var. *rubradiris*. *J. Antibiotics* **45:** 1313–1324.
4. Bechthold, A., J. K. Sohng, T. M. Smith, X. Chu, and H. G. Floss. 1995. Identification of *Streptomyces vialaceoruber* Tü22 genes involved in the biosynthesis of granaticin. *Mol. Gen. Genet.* **248:** 610–620.
5. Borisova, S. A., L. Zhao, C. E. MelanconIII, C. L. Kao, and H. W. Liu. 2004. Characterization of the glycosyltransferase activity of DesVII: Analysis of and implications for the biosynthesis of macrolide antibiotics. *J. Am. Chem. Soc.* **126:** 6534–6535.
6. Chen, H., M. G. Thomas, B. K. Hubbard, H. C. Losey, C. T. Walsh, and M. D. Burkart. 2000. Deoxysugars in glycopeptide antibiotics: Enzymatic synthesis of TDP-L-epivancosamine in chloroeremomycin biosynthesis. *Proc. Natl. Acad. Sci. USA* **97:** 11942–11947.
7. Chong, Y. H., J. M. Young, J. Y. Kim, Y. Y. Lee, K. S. Park, J. H. Cho, H. J. Kwon, J. W. Suh, and Y. H. Lim. 2006. *S*-Adenosyl-L-methionine analogues to enhance the production of actinorhodin. *J. Microbiol. Biotechnol.* **16:** 1154–1157.
8. Chung, Y. S., D. H. Kim, W. M. Seo, H. C. Lee, K. Liou, T. J. Oh, and J. K. Sohng. 2007. Enzymatic synthesis of dTDP-4-amino-4,6-dideoxy-D-glucose using GerB (dTDP-4-keto-6-deoxy-D-glucose aminotransferase). *Carbohydr. Res.* **342:** 1412–1418.
9. Dax, S. L. 1997. *Antibacterial Chemotherapeutic Agents*, 1st Ed. Blackie Academic & Professional, London.
10. Demain, A. L. 1999. Pharmaceutically active secondary metabolites of microorganisms. *Appl. Microbiol. Biotechnol.* **52:** 455–463.
11. Doumith, M., R. Legrand, C. Lang, J. A. Salas, and M. C. Raynal. 1999. Interspecies complementation in *Saccharopolyspora erythraea*: Elucidation of the function of *oleP1*, *oleG1*, and *oleG2* from the oleandomycin biosynthetic gene cluster of *Streptomyces antibioticus* and generation of new erythromycin derivatives. *Mol. Microbiol.* **34:** 1039–1048.
12. Elchert, B., J. Li, J. Wang, Y. Hui, R. Rai, R. Ptak, P. Ward, J. Y. Takemoto, M. Bensaci, and C. W. Chang. 2004. Application of the synthetic aminosugars for glycodiversification: Synthesis and antimicrobial studies of pyranmycin. *J. Org. Chem.* **69:** 1513–1523.
13. Fu, X., C. Albermann, C. Zhang, and J. S. Thorson. 2005. Diversifying vancomycin *via* chemoenzymatic strategies. *Org. Lett.* **7:** 1513–1515.
14. Graninger, M., B. Nidetzky, D. E. Heinrichs, C. Whitfield, and P. Messner. 1999. Characterization of dTDP-4-dehydrorhamnose 3,5-epimerase and dTDP-4-dehydrorhamnose reductase, required for dTDP-L-rhamnose biosynthesis in *Salmonella enterica* serovar Typhimurium LT2. *J. Biol. Chem.* **274:** 25069–25077.
15. Han, J. M., S. M. Kim, H. J. Lee, and J. C. Yoo. 2007. Cloning and expression of glucose-1-phosphate thymidylyltransferase gene (*schS6*) from *Streptomyces* sp. SCC-2136. *J. Microbiol. Biotechnol.* **17:** 685–690.
16. He, X. M. and H. W. Liu. 2002. Formation of unusual sugars: Mechanistic studies and biosynthetic applications. *Annu. Rev. Biochem.* **71:** 701–754.
17. Hoeksema, H. and J. C. Knight. 1975. The production of dihydrospectinomycin by *Streptomyces spectabilis*. *J. Antibiot.* **28:** 240–241.
18. Hong, J. S., S. H. Park, C. Y. Choi, J. K. Sohng, and Y. J. Yoon. 2004. New olivosyl derivatives of methymycin/pikromycin from an engineered strain of *Streptomyces venezuelae*. *FEMS Microbiol. Lett.* **238:** 391–399.

19. Hong, J. S., S. J. Park, N. Parajuli, S. R. Park, H. S. Koh, W. S. Jung, C. Y. Choi, and Y. J. Yoon. 2007. Functional analysis of *desVIII* homologues involved in glycosylation of macrolide antibiotics by interspecies complementation. *Gene* **386:** 123–130.
20. Hong, J. S., W. S. Jung, S. K. Lee, W. S. Koh, H. S. Park, S. J. Park, Y. S. Kim, and Y. J. Yoon. 2005. The role of a second protein (DesVIII) in glycosylation for the biosynthesis of hybrid macrolide antibiotics in *Streptomyces venezuelae*. *J. Microbiol. Biotechnol.* **15:** 640–645.
21. Hooper, D. C., J. S. Wolfson, G. L. McHugh, M. B. Winters, and M. N. Swartz. 1982. Effects of novobiocin, coumermycin A1, clorobiocin, and their analogs on *Escherichia coli* DNA gyrase and bacterial growth. *Antimicrob. Agents Chemother.* **22:** 662–671.
22. Huang, F., S. F. Haydock, T. Mironenko, D. Spiteller, Y. Li, and J. B. Spencer. 2005. The neomycin biosynthetic gene cluster of *Streptomyces fradiae* NCIMB 8233: Characterization of an aminotransferase involved in the formation of 2-deoxystreptamine. *Org. Biomol. Chem.* **3:** 1410–1419.
23. Huang, F., D. Spiteller, N. A. Koorbanally, Y. Li, N. M. Llewellyn, and J. B. Spencer. 2007. Elaboration of neosamine rings in the biosynthesis of neomycin and butirosin. *Chembiochem* **8:** 283–288.
24. Jaishy, B. P., S. K. Lim, I. D. Yoo, J. C. Yoo, J. K. Sohng, and D. H. Nam. 2006. Cloning and characterization of a gene cluster for the production of polyketide macrolide dihydrochalcomycin in *Streptomyces* sp. KCTC 0041BP. *J. Microbiol. Biotechnol.* **16:** 764–770.
25. Jo, Y. Y., S. H. Kim, Y. Y. Yang, C. M. Kang, J. K. Sohng, and J. W. Suh. 2003. Functional analysis of spectinomycin biosynthetic genes from *Streptomyces spectabilis* ATCC27741. *J. Microbiol. Biotechnol.* **13:** 906–911.
26. Jung, W. S., A. R. Han, J. S. Hong, S. R. Park, C. Y. Choi, J. W. Park, and Y. J. Yoon. 2007. Bioconversion of 12-, 14-, and 16-membered ring aglycones to glycosylated macrolides in an engineered strain of *Streptomyces venezuelae*. *Appl. Microbiol. Biotechnol.* **76:** 1373–1381.
27. Kang, Y. B., Y. H. Yang, K. W. Lee, S. G. Lee, J. K. Sohng, H. C. Lee, K. Liou, and B. G. Kim. 2006. Preparative synthesis of dTDP-L-rhamnose through combined enzymatic pathways. *Biotechnol. Bioeng.* **93:** 21–27.
28. Kharel, M. K., D. B. Basnet, H. C. Lee, K. Liou, Y. H. Moon, J. J. Kim, J. S. Woo, and J. K. Sohng. 2004. Molecular cloning and characterization of a 2-deoxystreptamine biosynthetic gene cluster in gentamicin-producing *Micromonospora echinospora* ATCC 15835. *Mol. Cells* **18:** 71–78.
29. Kharel, M. K., D. B. Basnet, H. C. Lee, K. Liou, J. S. Woo, B. G. Kim, and J. K. Sohng. 2004. Isolation and characterization of the tobramycin biosynthetic gene cluster from *Streptomyces tenebrarius*. *FEMS Microbiol. Lett.* **230:** 185–190.
30. Kharel, M. K., B. Subba, D. B. Basnet, J. S. Woo, H. C. Lee, K. Liou, and J. K. Sohng. 2004. A gene cluster for biosynthesis of kanamycin from *Streptomyces kanamyceticus*: Comparison with gentamicin biosynthetic gene cluster. *Arch. Biochem. Biophys.* **429:** 204–214.
31. Kharel, M. K., B. Subba, H. C. Lee, K. Liou, J. S. Woo, and J. K. Sohng. 2003. An approach for cloning biosynthetic genes of 2-deoxystreptamine-containing aminocyclitol antibiotics: Isolation of a biosynthetic gene cluster of tobramycin from *Streptomyces tenebrarius*. *Biotechnol. Lett.* **25:** 2041–2047.
32. Khosla, C. and R. J. X. Zawada. 1996. Generation of polyketide libraries *via* combinatorial biosynthesis. *Trends Biotechnol.* **14:** 2465–2497.
33. Kim, B. C., J. M. Lee, J. S. Ahn, and B. S. Kim. 2007. Cloning, sequencing, and characterization of the pradimicin biosynthetic gene cluster of *Actinomadura hibisca* P157-2. *J. Microbiol. Biotechnol.* **17:** 830–839.
34. Kim, C. Y., H. J. Park, and E. S. Kim. 2006. Functional dissection of sigma-like domain in antibiotic regulatory gene, *afsR2* in *Streptomyces lividans*. *J. Microbiol. Biotechnol.* **16:** 1477–1480.
35. Kim, K. P., C. S. Shin, S. J. Lee, J. H. Kim, J. M. Young, Y. Y. Lee, J. H. Ahn, J. W. Suh, and Y. G. Lim. 2006. Proteomes induced by *S*-adenosyl-L-methionine in *Streptomyces coelicolor* A3(2). *J. Microbiol. Biotechnol.* **16:** 799–803.
36. Kudo, F., M. Numakura, H. Tamegai, H. Yamamoto, T. Eguchi, and K. Kakinuma. 2005. Extended sequence and functional analysis of the butirosin biosynthetic gene cluster in *Bacillus circulans* SANK 72073. *J. Antibiot.* **58:** 373–379.
37. Kudo, F., Y. Yamamoto, K. Yokoyama, T. Eguchi, and K. Kakinuma. 2005. Biosynthesis of 2-deoxystreptamine by three crucial enzymes in *Streptomyces fradiae* NBRC 12773. *J. Antibiot.* **58:** 766–774.
38. Lamichhane, J., K. Liou, H. C. Lee, C. G. Kim, and J. K. Sohng. 2006. Functional characterization of ketoreductase (*rubN6*) and aminotransferase (*rubN4*) genes in the gene cluster of *Streptomyces achromogenes* var. *rubradiris*. *Biotechnol. Lett.* **28:** 545–553.
39. Langenhan, J. M., N. R. Peters, I. A. Guzei, F. M. Hoffmann, and J. S. Thorson. 2005. Enhancing the anticancer properties of cardiac glycosides by neoglycorandomization. *Proc. Natl. Acad. Sci. USA* **102:** 12305–12310.
40. Leadly, P. F. 1997. Combinatorial approaches to polyketide biosynthesis. *Curr. Opin. Chem. Biol.* **1:** 335–341.
41. Lee, H. C., 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 (*gerD*) from *Streptomyces* sp. GERI-155. *Mol. Cells* **17:** 274–280.
42. Lee, H. C., J. K. Sohng, H. J. Kim, D. H. Nam, C. N. Seong, J. M. Han, and J. C. Yoo. 2004. Cloning, expression, and biochemical characterization of dTDP-glucose 4,6-dehydratase gene (*gerE*) from *Streptomyces* sp. GERI-155. *J. Microbiol. Biotechnol.* **14:** 576–583.
43. Lee, S. K., J. W. Park, J. W. Kim, W. S. Jung, S. R. Park, C. Y. Choi, E. S. Kim, B. S. Kim, J. S. Ahn, D. H. Sherman, and Y. J. Yoon. 2006. Neopikromycin and novapikromycin from the pikromycin biosynthetic pathway of *Streptomyces venezuelae*. *J. Nat. Prod.* **69:** 847–849.

44. Lee, S. K., J. W. Park, S. R. Park, J. S. Ahn, C. Y. Choi, and Y. J. Yoon. 2006. Hydroxylation of indole by PikC cytochrome P450 from *Streptomyces venezuelae* and engineering its catalytic activity by site-directed mutagenesis. *J. Microbiol. Biotechnol.* **16:** 974–978.
45. Lee, Y. Y., J. M. Young, H. J. Kwon, J. W. Suh, J. Y. Kim, Y. H. Chong, and Y. H. Lim. 2006. AdoMet derivatives induce the production of actinorhodin in *Streptomyces coelicolor*. *J. Microbiol. Biotechnol.* **16:** 965–968.
46. Litovchick, A., A. Lapidot, M. Eisenstein, A. Kalinkovich, and G. Borkow. 2001. Neomycin B-arginine conjugate, a novel HIV-1 Tat antagonist: Synthesis and anti-HIV activities. *Biochemistry* **40:** 15612–15623.
47. Lu, W., C. Leimkuhler, G. J. Jr Gatto, R. G. Kruger, M. Oberthur, D. Kahne, and C. T. Walsh. 2005. AknT is an activating protein for the glycosyltransferase AknS in L-aminodeoxysugar transfer to the aglycone of aclacinomycin A. *Chem. Biol.* **12:** 527–534.
48. Madduri, K. and C. R. Hutchinson. 1995. Functional characterization and transcriptional analysis of the *dnrR1* locus, which controls daunorubicin biosynthesis in *Streptomyces peucetius*. *J. Bacteriol.* **177:** 1208–1215.
49. Maharjan, J., K. Liou, H. C. Lee, C. G. Kim, J. J. Lee, J. C. Yoo, and J. K. Sohng. 2003. Functional identification of *rub52* gene involved in the biosynthesis of rubradirin. *Biotechnol. Lett.* **25:** 909–915.
50. Mason, D. J., A. Dietz, and R. M. Smith. 1961. Actinospectacin, a new antibiotic. I. Discovery and biological properties. *Antibiot. Chemother.* **11:** 118–122.
51. Melancon, C. E. III, H. Takahashi, and H. W. Liu. 2004. Characterization of TylM3/TylM2 and MydC/MycB pairs required for efficient glycosyltransfer in macrolide antibiotic biosynthesis. *J. Am. Chem. Soc.* **126:** 16726–16727.
52. Newman, D. J., G. M. Cragg, and K. M. Snader. 2003. Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Prod.* **66:** 1022–1037.
53. Oh, J., S. G. Lee, B. G. Kim, J. K. Sohng, K. Liou, and H. C. Lee. 2003. One-pot enzymatic production of dTDP-4-keto-6-deoxy-D-glucose from dTMP and glucose-1-phosphate. *Biotechnol. Bioeng.* **84:** 452–458.
54. Park, N. S., H. J. Park, K. B. Han, and E. S. Kim. 2006. Heterologous expression of novel cytochrome P450 hydroxylase genes from *Sebekia benihana*. *J. Microbiol. Biotechnol.* **16:** 295–298.
55. Poulsen, S. M., C. Kofoed, and B. Vester. 2000. Inhibition of the ribosomal peptidyl transferase reaction by the mycarose moiety of the antibiotics carbomycin, spiramycin and tylosin. *J. Mol. Biol.* **304:** 471–481.
56. Rajkarnikar, A., H. J. Kwon, Y. W. Ryu, and J. W. Suh. 2007. Two threonine residues required for role of AfsKav in controlling morphogenesis and avermectin production in *Streptomyces avermitilis*. *J. Microbiol. Biotechnol.* **17:** 1563–1567.
57. Rodriguez, L., I. Aguirrezabalaga, N. Allende, A. F. Brana, C. Mendez, and J. A. Salas. 2002. Engineering deoxysugar biosynthetic pathways from antibiotic-producing microorganisms. A tool to produce novel glycosylated bioactive compounds. *Chem. Biol.* **9:** 721–729.
58. 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 a dTDP-deoxyglucose epimerase (*gerF*) gene from *Streptomyces* sp. GERI-155. *Biotechnol. Lett.* **26:** 185–191.
59. Sohng, J. K., T. J. Oh, J. H. Cha, J. J. Hahn, J. W. Kim, J. W. Suh, and H. C. Lee. 2001. Cloning and identification of a gene cluster in *Streptomyces spectabilis* - spectinomycin producer. *J. Biochem. Mol. Biol. Biophys.* **5:** 209–218.
60. Sohng, J. K., T. J. Oh, and C. G. Kim. 1998. Method for cloning biosynthetic genes of secondary metabolites including deoxysugar from Actinomycetes. *J. Biochem. Mol. Biol.* **31:** 475–483.
61. Sohng, J. K., T. J. Oh, J. J. Lee, and C. G. Kim. 1997. Identification of a gene cluster of biosynthetic genes of rubradirin substructures in *S. achromogenes* var. *rubradiris* NRRL 3061. *Mol. Cells* **7:** 674–681.
62. Solenberg, P. J., P. Matsushima, D. R. Stack, S. C. Wilkie, R. C. Thompson, and R. H. Baltz. 1997. Production of hybrid glycopeptide antibiotics *in vitro* and in *Streptomyces toyocaensis*. *Chem. Biol.* **4:** 195–202.
63. Steffensky, M., A. Muhlenweg, Z. X. Wang, S. M. Li, and L. Heide. 2000. Identification of the novobiocin biosynthetic gene cluster of *Streptomyces spheroides* NCIB 11891. *Antimicrob. Agents Chemother.* **44:** 1214–1222.
64. Subba, B., M. K. Kharel, H. C. Lee, K. Liou, B. G. Kim, and J. K. Sohng. 2005. The ribostamycin biosynthetic gene cluster in *Streptomyces rebosidificus*: Comparison with butirosin biosynthesis. *Mol. Cells* **20:** 90–96.
65. Subba, B., N. P. Kurumbang, Y. S. Jung, Y. J. Yoon, H. C. Lee, K. Liou, and J. K. Sohng. 2007. Production of aminoglycoside in non-aminoglycoside producing *Streptomyces lividans*. *Bioorg. Med. Chem. Lett.* **17:** 1892–1896.
66. Thuy, M. L., M. K. Kharel, R. Lamichhane, H. C. Lee, J. W. Suh, K. Liou, and J. K. Sohng. 2005. Expression of 2-deoxy-*scyllo*-inosose synthase (*kanA*) from kanamycin gene cluster in *Streptomyces lividans*. *Biotechnol. Lett.* **27:** 465–470.
67. Thuy, T. T., H. C. Lee, C. G. Kim, L. Heide, and J. K. Sohng. 2005. Functional characterizations of *novWUS* involved in novobiocin biosynthesis from *Streptomyces spheroides*. *Arch. Biochem. Biophys.* **436:** 161–167.
68. Thuy, T. T., K. Liou, T. J. Oh, D. H. Kim, D. H. Nam, J. C. Yoo, and J. K. Sohng. 2007. Biosynthesis of dTDP-6-deoxy-beta-D-allose, biochemical characterization of dTDP-4-keto-6-deoxyglucose reductase (*gerK1*) from *Streptomyces* sp. KCTC 0041BP. *Glycobiology* **17:** 119–126.
69. Tok, J. B., L. J. Dunn, and R. C. Des Jean. 2001. Binding of dimeric aminoglycosides to the HIV-1 rev responsive element (RRE) RNA constructs. *Bioorg. Med. Chem. Lett.* **11:** 1127–1131.
70. Truman, A. W., F. Huang, N. M. Llewellyn, and J. B. Spencer. 2007. Characterization of the enzyme BtrD from *Bacillus circulans* and revision of its functional assignment in the biosynthesis of butirosin. *Angew. Chem. Int. Ed. Engl.* **46:** 1462–1464.

71. Tsuchizaki, N., K. Ishino, F. Saito, J. Ishikawa, M. Nakajima, and K. Hotta. 2006. Trends of Arbekacin-resistant MRSA strains in Japanese hospitals (1979 to 2000). *J. Antibiot.* **59:** 229–233.
72. Waldron, C., P. Matsushima, P. R. Jr. Rosteck, 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.
73. Weber, J. M., J. O. Leung, S. J. Swanson, K. B. Ikler, and J. B. McAlpine. 1991. An erythromycin derivative produced by targeted gene disruption in *Saccharopolyspora erythraea*. *Science* **252:** 114–117.
74. Wehrli, W. 1977. Ansamycins. Chemistry, biosynthesis and biological activity. *Top. Curr. Chem.* **72:** 21–49.
75. Xue, Y., L. Zhao, H. W. Liu, and D. H. Sherman. 1998. A gene cluster for macrolide antibiotic biosynthesis in *Streptomyces venezuelae*: Architecture of metabolic diversity. *Proc. Natl. Acad. Sci. USA* **95:** 12111–12116.
76. Zhang, G., J. Shen, H. Cheng, L. Zhu, L. Fang, S. Luo, M. T. Muller, G. E. Lee, L. Wei, Y. Du, D. Sun, and P. G. Wang. 2005. Syntheses and biological activities of rebeccamycin analogues with uncommon sugars. *J. Med. Chem.* **48:** 2600–2611.
77. Zhao, X. Q., Y. Y. Jin, H. J. Kwon, Y. Y. Yang, and J. W. Suh. 2006. *S*-Adenosylmethionine (SAM) regulates antibiotic biosynthesis in *Streptomyces* spp. in a mode independent of its role as a methyl donor. *J. Microbiol. Biotechnol.* **16:** 927–932.
78. Zhu, L., X. Cao, W. Chen, G. Zhang, D. Sun, and P. G. Wang. 2005. Syntheses and biological activities of daunorubicin analogs with uncommon sugars. *Bioorg. Med. Chem.* **13:** 6381–6387.