Phosphopantetheinyl transferases (PPTases) are a superfamily of essential enzymes required for the synthetic processes of many compounds including fatty acid, polyketide, and nonribosomal peptide metabolites. These enzymes activate carrier proteins in specific biosynthetic pathways via the transfer of a phosphopantetheinyl moiety to a serine residue in the conserved motif of carrier proteins. Since many Actinomycetales microorganisms produce a number of polyketide and nonribosomal peptide metabolites, the distribution of PPTase genes was investigated in these microorganisms. PPTases were found in bacterial protein databases using a hidden Markov model search with the PF01648 (4′-phosphopantetheinyl transferase superfamily) model. Actinomycetales microorganisms harbor several genes encoding AcpS-type and Sfp-type PPTases in individual genomes, many of which were associated with the biosynthetic gene cluster for polyketide or nonribosomal peptide metabolites. The properties of these PPTases were evaluated in the heterologous expression system using the biosynthetic gene clusters and genes encoding PPTases found in the present study. Sfp-type PPTases were classified into two subgroups, and although the substrate specificities of the enzymes in one subgroup were wide, the catalytic activities of enzymes in the other subgroup were low. SAV_1784 of Streptomyces avermitilis possessed the most characteristic broad-range activity against several type I polyketide synthases and nonribosomal peptide synthetases.

Distribution and functional analysis of the phosphopantetheinyl transferase superfamily in Actinomycetales microorganisms

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Microbial natural products have various structures and biological functions. Some of these products are used as clinically important drugs including antibacterial, antifungal, antitumor, antiviral, antimetadote, and immunosuppressive agents (1). Macrocyclic polyketide compounds (macrolides or macroyclic lactones) synthesized by type I (modular) polyketide synthases (PKSs) and peptide compounds synthesized by nonribosomal peptide synthetases (NRPSs) have diverse structures and various biological activities (2). The polyketide compounds erythromycin, amphotericin B, and avermectin are used in the treatment of bacterial, fungal, and nematode infectious diseases, respectively, while the peptide compounds β-lactam compound (cephamycin C) and actinomycin D are used as antibacterial and antitumor agents, respectively.

Phosphopantetheinyl transferases (PPTases) catalyze posttranslational modifications to carrier proteins in fatty acid synthases (FASs), PKSs, and NRPSs (3). Carrier proteins are either integrated into these synthases (type I) or are discrete subunits (type II) and, depending on the nature of the attached intermediate, are called acyl carrier proteins (ACPs) or peptidyl carrier proteins (PCPs), respectively (4–8). Each biosynthetic pathway may encode several carrier proteins, the number of which typically correlates with the length of the final product. Phosphopantetheinylation by PPTases occurs by the transfer of the 4′-phosphopantetheine prosthetic group from CoA to a conserved serine residue in the carrier proteins, converting the proteins from their inactive “apo” forms to their active “holo” forms (9). The 4′-phosphopantetheine arm on holo form carrier proteins has two functions for the elongation of the acyl or peptidyl chain on FAS, PKS, or NRPS. The free thiol group of the phosphopantetheine acts as a nucleophile for a covalent connection by a nucleophilic reaction for the intermediates to form an acyl or peptidyl thioester. The length and flexibility of this moiety assists in the relocation of intermediates between spatially distinct modules of the complex (10, 11).

PPTases have been extensively classified into three structural groups, which correlate with their substrate specificities and lengths (9, 12). The first group is “AcpS-type” PPTases. These PPTases are ~115 amino acids in size, function as homotrimers, and are found in most microorganisms for specific modifications to discrete carrier proteins as an essential component of fatty acid synthesis (13–15). Some of these enzymes activate a wide range of substrates in vitro (carrier proteins of type I and II PKSs) (16–18). The second group is “Sfp-type” PPTases, which

Significance

Actinomycetales microorganisms are a rich source of secondary metabolites, and their genomes contain many biosynthetic gene clusters for metabolites including polyketide and peptide compounds synthesized by type I polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs). Genes encoding Sfp-type phosphopantetheinyl transferase (PPTase), which modifies the “apo” form to “holo” form carrier protein on type I PKSs and NRPSs, were widely distributed in Actinomycetales microorganisms, which is similar to the distribution of biosynthetic gene clusters for polyketide and peptide compounds synthesized by type I PKSs and NRPSs, respectively. Some actinomycete PPTases exhibited characteristic broad-range activities against several type I PKSs and NRPSs. These PPTases will be useful for the coexpression of several biosynthetic gene clusters including type I PKSs and NRPSs.
are classified by the prototype PPTase from *Bacillus subtilis*, Sfp. This PPTase is required for the activation of carrier proteins incorporated within the biosynthetic pathway that are responsible for the production of surfactin (11). These PPTases are monomorphic (19) and approximately twice the size of AcpS-type enzymes (∼230 amino acids), which suggests that they evolved by gene duplication from an AcpS ancestor (12). This family includes PPTase involved in cyanobacterial heterocyst differentiation (20). Sfp-type PPTases are considered to be optimized for carrier proteins of secondary metabolism, and carrier proteins on the multifunctional enzymes, type-I PKS and NRPS, are mainly activated by these PPTases (21). The third type of PPTase is incorporated as a domain at the C terminus of the α-subunit of fungal FASs, at which it catalyzes the in cis autophosphopantetheinylation of the carrier protein at the N terminus of the same polypeptide (22). PPTases integrated into the fungal FKS have been reported (23), but the reports of the integrated-type PPTases from bacterial origins were quite few. Type I PKSs carrying the PPTase domain at the C-terminal region had been found in *Saccharopolyspora erythraea* NRRRL 2338 (SACE_2433) (24) and *Streptomyces avermitilis* MA-4680 (SADV_7361) (25); however, the products synthesized by these type I PKSs have not been identified.

Microorganisms possess multiple PPTases, such as AcpS-type and Sfp-type PPTases. The *B. subtilis* genome encoded AcpS and Sfp-like PPTases. The genome of *Escherichia coli* had three PPTases: AcpS (for FAS), EntD (for the synthesis of NRPS–siderophore enterobactin; ref. 26), and YhtU (uncharacterized PPTase; refs. 9 and 27). These enzymes act independently in distinct pathways and display contrasting specificities for carrier proteins. Sfp-type PPTases are proposed to have arisen via gene duplication (enzyme domain) and subsequent divergence from ancestral AcpS-like PPTases. Despite the absolute requirement for PPTases in a wide range of important and fundamental biosynthetic pathways, these PPTases have remained elusive due to their low sequence identities and lack of proximity to their respective biosynthetic clusters. *Actinomycetales* microorganisms are known as producers of a number of secondary metabolites, including polyketide and peptide compounds. Since the synthetic process of these polyketide and peptide compounds is involved in type I PKSs or NRPSs, the producing microorganisms will harbor specific PPTases for carrier proteins on type I PKSs or NRPSs.

Several AcpS-type PPTases have been characterized by the enzymatic conversion of the apo form to the holo form of carrier proteins in vitro (7, 12, 16, 18), and, in many cases, the characteristics of Sfp-type PPTases were also obtained by an in vitro reaction using an artificial small polypeptide containing a carrier protein domain from type I PKSs or NRPSs (11, 12, 24). The experimental characteristics of Sfp-type PPTases using native type I PKSs and NRPSs were not reported to date. We examined the characteristics of four Sfp-type PPTases of the industrial producer *S. avermitilis* MA-4680 using deletion mutants of these genes and a heterologous expression system based on *E. coli* proteomes (16). Phosphopantetheinylation was evaluated by the enzymatic conversion of the apo form to the holo form of carrier proteins on type I PKSs or NRPSs (11, 12, 16, 18), and, in many cases, the characteristics of Sfp-type PPTases were further classified into two subgroups indicated in the Sfp-type PPTases of *Cyanobacteria* and *Proteobacteria* (28). Sfp-type PPTases from *Actinomycetales* microorganisms also belong to these two groups, which have the conserved motifs [ILV][G][ILV][D][AILV][EQ]...33–41 aa...WxxKEAxK (WxxKEAxK subtype) and [ILV][G][ILV][D][AILV]...33–46 aa...FxKEAx[F]K (FxKEAxK subtype), respectively (SI Appendix, Fig. S5 A and B, I). The alignment of the amino acid sequences of FxKEAxK subtype PPTases indicated that these PPTases have more conserved motifs upstream of the two conserved motifs described above. Two other conserved motifs are Rx[AC]Ax[...17–18 aa...Px[FW]Pxx[AG][AS][ST]H (SI Appendix, Fig. S1 B, I). The second motifs were found in *Actinomycetales* microorganisms, *E. coli* K-12 substr. MG1655, and *Pseudomonas putida* KT 2440 (also *P. aeruginosa* PAO1), whereas the corresponding PPTases were not found in *Cyanobacteria*. WxKEAxK subtype PPTases were more widely distributed, and the total number of this subtype of PPTases was approximately twice that of FxKEAxK subtype PPTases (Fig. 1). Although PPTases incorporated as a domain at the C terminus of type I PKS had only been reported in erythromycin-producing *S. erythraea* NRRRL 2338 and avermectin-producing *S. avermitilis* MA-4680 (24), an additional six PPTases were found as a domain at the C terminus of undefined type I PKSs from *Actinosynnema mirum* DSM 43827 (Amir_2494), *Amycolatopsis mediterranei* U32 (AMED 4605), *S. violaceusniger* Tu 4113 (Strvi_6300), *S. collinus* Tu 365 (B446 (6810), *S. olivochromogenes* (accession no. WP_079065395), and *S. mirabilis* (accession no. WP_075031111). The products...
generated by these type I PKSs were unidentified; however, this type of PPTase incorporated into type I PKS may be widely distributed in Actinomycetales microorganisms. PPTases incorporated as a domain at the C terminus of fungal FAs were classified as Acps-type PPTases, whereas PPTases on the C terminus of type I PKSs of Actinomycetales microorganisms were classified as Sfp-type PPTases (Fig. 1).

**Characteristics of Four Sfp-Type PPTases in S. avermitilis**. The anthelmintic macrolide lactone, avermectin, producer S. avermitilis (25) harbors one AcpS-type, four Sfp-type, and one PKS integrated-type PPTases (SI Appendix, Table S1). The four Sfp-type PPTases were classified into the above FxkEAsxk subtype (SAV_1748, PptA2 and SAV_2905, PptA1) and WxkEAsxk subtype (SAV_3193, PptA3 and SAV_3637, PptA4), respectively. Three genes encoding PptA1, PptA3, and PptA4 were located in the region flanking the gene cluster including type I PKS (oligomycin) and NRPS genes (nrps1 and nrps2; the products were not identified by these NRPSs), respectively (avermitilis.ls.kitasato-u.ac.jp/gview/). The three genes, pptA1, pptA2, and pptA4, form an operon with a gene encoding phosphoesterase (possibly 3′,5′-ADP phosphatase; the reaction with the apo-carrying protein and CoA by PPTase generates a holocomponent and 3′,5′-ADP. This phosphoesterase will catalyze the generation of 5′-AMP from 5′-ADP (31)). S. avermitilis produces lactone-type polyketide compounds: filipins, avermectins, and oligomycin, which are synthesized by type I PKSs (29). Our initial experiments indicated that the phosphopantetheinyltransfer of apo-carrying proteins on filamentin and avermectin PKSs was controlled by PptA1 and PptA2 because the single-deletion mutants, pptA1 and pptA2, produced filipins and avermectins, whereas double-deletion mutants completely failed to produce either of these polyketide compounds (SI Appendix, Fig. S2).

Genetically engineered S. avermitilis SUKA series are suitable for the heterologous expression of biosynthetic gene clusters for polyketides and peptide compounds that are synthesized by type I PKSs and NRPSs (29-31). Phosphopantetheinyltransferation by the four PPTases of S. avermitilis were examined in the production of actinomycin X1 (NRPS), cephemycin C (NRPS), chloramphenicol (NRPS), indigoidine (NRPS), lactacystin (NRPS/PKS hybrid), pikromycin (type I PKS), and nemadectin (type I PKS). We constructed series of pptA-deletion mutants (single, double, triple, and quadruple deletions) in genetically engineered S. avermitilis SUKA38 carrying the hygromycin B resistance gene (hph from S. hygroscopicus ATCC 39263). All exogenous biosynthetic gene clusters were expressed on the linear plasmid SAP1 of S. avermitilis. Since the SAP1 plasmid possesses the transfer function, it was easy to introduce into each deletion mutant by simple mating between SUKA37 carrying the erythromycin resistance gene (ermE of Sa. erythraea NRRL 2338) and vector containing the biosynthetic gene cluster and SUKA38 carrying hygromycin B resistance gene (SI Appendix, Fig. S3).

The production of the deep blue pigment, indigoidine, was completely terminated in the mutants lacking pptA2 (Fig. 2, IV; Δ1, Δ2, Δ3, Δ4, Δ12Δ3, Δ12Δ4, and Δ12Δ3Δ4). The production of actinomycin X1 and chloramphenicol was markedly reduced in pptA2-deletion mutants, and the productivities of both compounds were completely terminated in mutants lacking the pptA1 and pptA2 genes (Fig. 2, I; Δ1, Δ2, Δ1Δ2Δ3, Δ1Δ2Δ4, and Δ12Δ3Δ4). Another NRPS compound, cephemycin C, was normally produced in the single-deletion mutants of pptA1 or pptA2, whereas production was completely terminated in double-deletion mutants of pptA1 and pptA2 (Fig. 2, II; Δ1Δ2, Δ1Δ2Δ3, Δ1Δ2Δ4, and Δ12Δ3Δ4). The productivities of the NRPS/PKS hybrid compound, lactacycin, and macrocyclic lactone compounds, nemadectin, were not affected in the single-deletion mutants, but were completely terminated in the mutants lacking pptA1 and pptA2 (Fig. 2, V-VII; Δ1Δ2, Δ1Δ2Δ3, Δ1Δ2Δ4, and Δ12Δ3Δ4). Thus, PptA1 and PptA2 both have the ability to phosphopantetheinylate several exogenous type I PKSs and NRPSs. PptA2 appears to be preferable for modifying NRPSs later than type I PKSs. No metabolites were detected in mutants lacking at least two genes, pptA1 and pptA2. The quadruple pptA-deletion mutants carrying the biosynthetic gene cluster for chloramphenicol-deleted cmlL (cmlL encodes a Sfp-type PPTase) did not produce any antimicrobial substances (SI Appendix, Fig. S4) that was identical to 2-amino-3-(4-aminophenyl)propanal by an authentic sample prepared from p-amino-phenylalanine by organic synthesis. The NRPS, CmlP, for chloramphenicol biosynthesis consists of adenylation, PCP, and reductase domains (32). An aldehyde product may be generated from an important intermediate, p-amino-phenylalanine, by the reduction of the carboxylic acid residue.

**Fig. 2.** Productivities of polyketide and peptide metabolites ([I] actinomycin X1, [II] cephemycin C, [III] chloramphenicol, [IV] indigoidine, [V] lactacystin, [VI] pikromycin, and [VII] nemadectin) in various pptA-deletion mutants of S. avermitilis. Each metabolite was examined in engineered S. avermitilis SUKA38, A1 (pptA1), A2 (pptA2), A3 (pptA3), A4 (pptA4), A12 (pptA1 pptA2), A13 (pptA1 pptA3), A14 (pptA1 pptA4), A23 (pptA2 pptA3), A24 (pptA2 pptA4), A34 (pptA3 pptA4), A123 (pptA1 pptA2 pptA3), A124 (pptA1 pptA2 pptA4), A134 (pptA1 pptA3 pptA4), A234 (pptA2 pptA3 pptA4), A1234 (pptA1 pptA2 pptA3 pptA4) carrying the SAP1 vector containing the entire biosynthetic gene cluster. Productivity was calculated by at least three independent datasets, and vertical bars indicate SDs.
Since compounds synthesized through aminoglycoside biosynthesis, ribosomal peptide synthesis, or the type II or III PKS pathway do not require phosphopantetheinylation by Sfp-type PPTases, the production of these compounds will not be affected in mutants lacking all genes encoding Sfp type PPTases. The production of streptomycin (aminoglycoside; SI Appendix, Fig. S6), asukamycin (synthesized by type II PKS; SI Appendix, Fig. S7), phenolic lipids (synthesized by type III PKS; SI Appendix, Fig. S8), and thioestrepton (RiPPs: ribosomally synthesized and posttranslationally modified peptides; SI Appendix, Fig. S9) was not affected in quadruple pptA-deletion mutants because these biosynthetic processes do not require posttranslational modifications by Sfp-type PPTases. Discrete apo-carrier proteins in type II and III PKS systems would be modified by AcpS-type PPTase such as FAS system.

Evaluation of PPTases in Actinomycetales Microorganisms by a Heterologous Expression System Using Quadruple pptA-Deletion Mutants of S. avermitilis. The quadruple pptA-deletion mutants of S. avermitilis lack all genes encoding Sfp-type PPTases. Therefore, they are suitable for the evaluation of exogenous PPTase in a combination of quadruple pptA-deletion mutants and biosynthetic gene clusters for polyketide and peptide compounds synthesized by type I PKSs and NRPSs. Sfp of B. subtilis subsp. subtilis str. NCIB 3610 (accession no. ABV89947) was used for phosphopantetheinylation in the E. coli system, and its GC content of coding region is 46.67 GC mol%. A synthetic gene (62.96 GC mol%) encoding Sfp of B. subtilis subsp. subtilis NCIB 3610, the nucleotide sequence of which was optimized for S. avermitilis codon usage, was prepared (accession no. LC341265) for efficient translation in S. avermitilis and used as a positive control of the exogenous pptA gene. The expression level of each gene encoding exogenous PPTase by a native promoter was unknown. The expression levels of extrinsic PPTase gene were compared using its own promoter and a promoter optimized for expression in the production phase of S. avermitilis. Using quadruple pptA-deletion mutants of S. avermitilis SUKAS38 carrying the SAP1 vector containing entire biosynthetic gene clusters for cephemycin C and chloramphenicol (SI Appendix, Fig. S10). The productivity of the peptide compounds, cephemycin C and chloramphenicol, in quadruple pptA-deletion mutants were efficient under control of the pptA gene by an alternative promoter, P_{sav2794} (a promoter of the gene encoding secreted metalloprotease, SAV_2794; ref. 33). Chloramphenicol productivity under the expression of the gene encoding S. clavuligerus ATCC 27064 (clavulanic acid) was enhanced more by P_{sav2794} than the promoter of each pptA gene (SI Appendix, Fig. S10). Since the expression of the exogenous pptA gene using its own promoter was not suitable in S. avermitilis, all exogenous pptA genes were controlled under the optimized promoter P_{sav2794}. Exogenous PPTases (two subtypes of Sfp-type PPTases) of Actinomycetales microorganisms were chosen from peptide compound (generated by type I PKS) producer S. clavuligenus ATCC 27064 (cephemycin C) and S. venezuelae ATCC 10712 (chloramphenicol), polyketide compound (generated by type I PKS) producer S. erythraea RRL 2338 (erythromycin), and peptide/polyketide compounds (NRPS or type I PKS) nonproducer S. griseus IFO 13350 (streptomycin). S. lividans TK24 and S. albus J10174. Two PPTases from Pseudomonas aeruginosa PA01 and P. putida KT2440 were used as Proteobacteria PPTases.

The production of actinomycin X \textsubscript{1} in quadruple pptA-deletion mutants of S. avermitilis SUKAS38 was fully restored by SAV_1748 (PptA2), SAV_05145, XNR_5716, SCLAV_0102, SCLAV_2604, SVEN_0914, SVEN_5990, SVEN_0484, SGR_655, and Sfp. SAV_3637 (PptA3) did not have the ability to modify actinomycin NRPSs in the series of pptA-deletion mutants of S. avermitilis, whereas SAV_3637 (PptA4) had the ability to modify actinomycin NRPSs under the control of the P_{sav2794} promoter. Furthermore, PPTases from both Pseudomonas strains exhibited the ability to modify actinomycin NRPSs (Fig. 3, I). Cephemycin C productivity was restored in quadruple-deletion mutants by SAV_2905 (PptA1), SAV_1748 (PptA2), SLIV_05145, SAV_0910, XNR_5716, SACE_4001, SCLAV_0102, SCLAV_2604, SVEN_0914, SVEN_5990, SVEN_0484, SGR_655, and Sfp. PptA4 in S. avermitilis also had the ability to modify AcvS (cephemycin C NRPS; Fig. 3, II). Three genes encoding SAV_0910, SVEN_0914, and SVEN_0484 were located in the biosynthetic gene clusters for undecylprodigiosin, chloramphenicol, and an unknown NRPS-PKS hybrid, respectively. Both Pseudomonas PPTases also modified AcvS (Fig. 3, II). The production of chloramphenicol in the quadruple-deletion mutants was fully restored by SAV_2905 (PptA1), SAV_1748 (PptA2), SLIV_05145, SAV_0910, XNR_5716, SACE_4001, SCLAV_0102, SCLAV_2604, SVEN_0914, SVEN_5990, SVEN_0484, SGR_655, and Sfp. Chloramphenicol productivity was partially restored by SLIV_05145, SAV_0910, and XNR_5716 (Fig. 3, III). The production of chloramphenicol in the quadruple-deletion mutants was not completed by PptA3. A gene encoding SVEN_0914 was located in the gene cluster for the chloramphenicol biosynthesis of S. venezuelae ATCC 10740; however, SVEN_0484 and SVEN_5990 efficiently modified chloramphenicol NRPS (CmlP). The PPTase of P. putida KT2440 modified CmlP, whereas that of P. aeruginosa PA01 did not (Fig. 3, III). Indigoidine was synthesized by one NRPS enzyme, LbpA. Indigoidine productivity in quadruple-deletion mutants was restored by SAV_1748 (PptA2), SLIV_05145,
Lactacytin was generated by type I PKS and NRPS, a process in which three carrier proteins (two in type I PKS and one in NRPS) were phosphopantetheinylated by Sfp-type PPTase. The productivity of lactacytin in quadruple ppa-deletion mutants was fully restored by SAV_1748 (PptA2), SAV_5990, SGR_665, and Sfp. Lactacytin productivity was partially restored by SAV_2905 (PptA1), SLIV_05145, SLV_09190, SAV_5716, SCLAV_0102, SCLAV_2604, SAV_0484, SGR_665, SAV_0914, SAV_0484, and SGR_5185. Two Pseudomonas Sfp-type PPTases also restored lactacytin production (Fig. 3, IV). The structure of the antibacterial 14-membered macrolide pikromycin is similar to that of erythromycin. Pikromycin productivity in quadruple-deletion mutants was restored by SAV_2905 (PptA1), SAV_1748 (PptA2), SAV_5716, SCLAV_0102, SCLAV_2604, SAV_0484, SGR_665, PP_1183, and Sfp (Fig. 3, I, T). Neither PPTases of erythromycin-producing Sa. erythraea NRRL 2338 had the ability to convert the apo form to the holo form of pikromycin PKSs. Sa. erythraea NRRL 2338 possesses two genes encoding discrete Sfp-type PPTases, and previous studies reported that SACE_4001 plays a role in modifications to the apo form of CmlP and/or the metabolism of CmlM. As chromophore and holo forms of CmlP and CmlM are both modified by the same CmlP, the results indicate that Pikromycin productivity in quadruple-deletion mutants was restored by SAV_2905 (PptA1), SAV_1748 (PptA2), SLIV_05145, SAV_5716, SCLAV_0102, SCLAV_2604, SAV_0484, SGR_665, PP_1183, and Sfp. Restoration by SACE_4001, SAV_0914, SAV_0484, and SGR_5185 was weak. Although SACE_4001 has the ability to modify erythromycin apo form type I PKS, SACE_4001 does not recognize other types of I PPKs (pikromycin and nemadecin) exhibiting similar macrocyclic lactone biosynthesis as the substrates (Fig. 3, VII).

Discussion

Several Sfp-type PPTases were found not only in Cyanobacteria and Proteobacteria, but also in Actinomycetales microorganisms. Furthermore, the Sfp-type PPTase of Actinomycetales microorganisms were classified into two consensuses: the WxxKEAxxK and FxxKESxxK subtypes. These PPTases have two conserved motifs [ILV]G[ILV]D[AILV][EQ] and WxxKEAxxK or FxxKES[F]EK, which have been reported in PPTases in Eubacteria (28). These findings suggest that cyanobacterial and actinomycete PPTases have both evolved from the same ancestor. The catalytic activities against type I PKSs and/or type II NRPSs exhibited by the two above Sfp-type PPTases indicate that the evolution of type I PKSs and NRPSs was preferred. Genes encoding Sfp-type PPTases, except for SAV_1748 (PptA2), are located on the side flanking the biosynthetic gene cluster for type I PKS or NRPS. These results indicate that a gene encoding SAV_3637 was in a cryptic state in S. avermitilis, and SAV_3193 did not possess the ability to modify the type I PKSs or NRPSs examined.

Acyl or peptidyl chain elongation during polyketide or peptide synthesis is accomplished on the phosphopantetheinyl moiety of holo form carrier proteins (ACP or PCP) on type I PKSs or NRPSs. Accordingly, deletions of all genes encoding Sfp-type PPTases in the genome resulted in the failed production of these metabolites or their intermediates. Genes encoding Sfp-type PPTases, catalytic activities against type I PKSs and NRPSs were found for many FxxKESxxK subtype PPTases, but type I PKSs and NRPSs were preferred. Genes encoding SFP_1183 restored indigoidine pptA microorganisms were used for posttranslational modifications to this microorganism. Four genes were mainly expressed, and these two gene products were preferred. Genes encoding Sfp-type PPTases, except for SAV_1748 (PptA2), are located on the side flanking the biosynthetic gene cluster for type I PKS or NRPS. These results indicate that a gene encoding SAV_3637 was in a cryptic state in S. avermitilis, and SAV_3193 did not possess the ability to modify the type I PKSs or NRPSs examined.

Some biosynthetic gene clusters for polyketide and peptide compounds contained a gene encoding Sfp-type PPTase. Three out of four PPTases in S. avermitilis were located in the gene clusters described above. A gene encoding SAV_0914 (CmlL) was also located in the biosynthetic gene cluster for chloramphenicol. The activity of SAV_0914 was confirmed by heterologous expression using the entire gene cluster and its derivative containing the in-frame deletion of cmlL (SI Appendix, Fig. S4). The cmlL-deletion mutants of chloramphenicol-producing S. venezuelae ATCC 10712 still produced chloramphenicol, but at lower productivity (32). Other PPTases of S. venezuelae, SAV_0484 and SAV_5990 (JadM; ref. 34), exhibited the catalytic activity to modify CmlP. SAV_0484 was particularly modified by CmlP rather than SAV_0914 (CmlL). cmlL-deletion mutants of S. venezuelae still produced chloramphenicol because the apo form of CmlP was still converted to its holo form by SAV_0484 and SAV_5990. The genes for prenylation enzymes were present in the unknown NRPS-PKS hybrid cluster and the biosynthetic gene cluster for jadomycin, respectively.

A previous study reported that the AcpS-type PPTase (SCO4744) of S. coelicolor A3 (2) appeared to be extremely promiscuous in its
substrate specificity, accepting protein substrates from type I (Rat) and type II [ACP of E. coli and S. coelicolor A3 (2)] FASs as well as type I (norfosolic acid synthase) and type II (ACP of octaetecycline PKS) PKSs (18). However, these ACPs of type I FASs and PKSs were small artificial polypeptides; therefore, it remains unclear whether the enzyme catalyzes modifications to native giant type I FASs or PKSs. AcpS-type PTase, SAV-4964 (80% identity and 91% similarity to SCO4744), of S. avermitilis MA-4680 did not catalyze modifications to carrier proteins on the type I PKSs and NRPSs examined because S. avermitilus SUKA38 quadruple ptaA-deletion mutants carrying the biosynthetic gene cluster including type I PKS or NRPS genes never produced polyketide or peptide metabolites. AcpS-type PTases may modify the apio apo form carrier proteins of type I PKSs and NRPSs prepared by a recombinant technique; however, their carrier proteins on native enzymes will not be modified. The carrier proteins on native type I PKSs and NRPSs may be exclusively modified by Sfp-type PTAses. Based on the results obtained for several PTase activities under this heterologous expression system, not only SAV_1748 (PtaA2) but also Sfp (B. subtilis) possessed the most characteristic broad-range activity against the several type I PKSs and NRPSs examined. Although their modification activities were slightly weaker, SLIV_05145, SCLAIV_2604, and SGR_665 also exhibited relatively broad-range activity. The expression of these genes encoding Sfp-type PTases in the heterologous host may be useful for the preparation of the holo form of several type I PKSs and NRPSs.

Materials and Methods

Locus tags or accession numbers in figures are as follows: A. niger FAS, Aspergillus niger phosphopantetheine-protein transferase domain protein (accession no. OW29103); A. parasiticus FAS, A. parasiticus FAS alpha subunit (accession no. QBTGAI9); B. subtilis Sfp, Subtilis sp. subtilis str. NCIB 3610 (accession no. ABV89947); AMED, A. mediterranei U32; Amir, A. mirum DSM 43827; B446, S. collinus Tu 365; BSU, B. subtilis subsp. subtilis str. 168; Fusarium_sp FAS, Fusarium sp. NRRL 25184 FAS alpha subunit (accession no. AMD39096); MXAN, Myxococcus xanthus DK 1622; NP, E. coli str. K-12 substr. MG1655; Npun, Nostoc punctiforme PCC 73102; PA, P. aeruginosa PA01; PPT, P. putida KT2440; SACE, Sa. erythraea NRRL 2338; SAM23877, S. ambipolaris ATCC 2877; SAV, S. avermitilis MA-4680; SCAB, scabie 87;22; SCLAV, S. clavuligerus ATCC 27064; SCO, S. coelicolor A3 (2); SGR, S. griseus IFO 13350; Strop, Saltinispora tropica CBN-440; Strv, S. violaceusniger Tu 4113; SVEN, S. venezuelae ATCC 10712; and XNR, S. albus J1074.

Bacterial strains, growth conditions, bioinformatics, the construction of ptaA-deletion mutants, the introduction of biosynthetic gene clusters for secondary metabolites, and analytical conditions for secondary metabolites are described in SI Appendix, SI Materials and Methods.

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