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ORIGINAL ARTICLE

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Identification of a vitamin D_3-specific hydroxylase genes through actinomycetes genome mining

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Abstract We previously completed whole-genome sequencing of a rare actinomycete named *Sebekia benihana*, and identified the complete *S. benihana* cytochrome P450 complement (CYPome), including 21 cytochrome P450 hydroxylase (CYP), seven ferredoxin (FD), and four ferredoxin reductase (FDR) genes. Through targeted CYPome disruption, a total of 32 *S. benihana* CYPome mutants were obtained. Subsequently, a novel cyclosporine A region-specific hydroxylase was successfully determined to be encoded by a CYP-sb21 gene by screening the *S. benihana* CYPome mutants. Here, we report that *S. benihana* is also able to mediate vitamin D_3 (VD_3) hydroxylation. Among the 32 *S. benihana* CYPome mutants tested, only a single *S. benihana* CYP mutant, ΔCYP-sb3a, failed to show regio-specific hydroxylation of VD_3 to 25-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3. Moreover, the VD_3 hydroxylation activity in the ΔCYP-sb3a mutant was restored by CYP-sb3a gene complementation. Since all *S. benihana* FD and FDR disruption mutants maintained VD_3 hydroxylation activity, we conclude that CYP-sb3a, a member of the bacterial CYP107 family, is the only essential component of the in vivo regio-specific VD_3 hydroxylation process in *S. benihana*. Expression of the CYP-sb3a gene exhibited VD_3 hydroxylation in the VD_3 non-hydroxylating *Streptomyces coelicolor*, implying that the regio-specific hydroxylation of VD_3 is carried out by a specific P450 hydroxylase in *S. benihana*.

Keywords *Sebekia benihana* · Vitamin D_3 · Cytochrome P450 hydroxylation · Rare actinomycetes

Introduction

Actinomycetes, ubiquitous high G+C Gram-positive soil-dwelling bacteria, possess biosynthetic clusters of many valuable secondary metabolites, such as antibiotics and anticancer drugs, as well as various bioconversion enzymes, including cytochrome P450 hydroxylases (CYPs). In particular, a rare actinomycetes named *Sebekia benihana* has the outstanding capability to introduce a hydroxyl group at specific regions of various natural products, such as monensin, nigericin, and cyclosporine A [1–3]. CYP, a heme-containing monooxygenase enzyme, is one of the most important industrial enzymes, catalyzing the regio-specific hydroxylation of endogenous and xenobiotic compounds [4]. It is also involved in key steps of various bioprocesses, including the first step of the steroid hormone synthesis pathway and the bioconversion of cholesterol. In general, CYP is present in most living organisms and its electron transport partners, such as ferredoxin (FD) and ferredoxin reductase (FDR), are also required for the activation of CYP [5].

Vitamin D_3 (VD_3) is a prohormone that plays a crucial role in plasma calcium and phosphate homeostasis regulation, cell differentiation, proliferation, and immunomodulation. It has important effects for the treatment and prevention of various diseases, such as hypothyroidism, osteoporosis, and chronic renal failure [6–8]. VD_3 is

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synthesized from 7-dehydrocholesterol in skin exposed to UV light, and exists in a physiologically inactive form [9]. To perform its physiological functions, through binding the vitamin D receptor, VD_3 must be converted to 25-hydroxyvitamin D_3 ($25(OH)VD_3$) and 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2VD_3$). These two reactions are performed by CYPs in the liver and kidney, respectively [6, 10–14]. Therefore, patients who have liver or kidney dysfunctions are unable to synthesize $1\alpha,25(OH)_2VD_3$ and have to take active forms of VD_3 from external sources.

The molecule $1\alpha,25(OH)_2VD_3$ is commonly synthesized by chemical methods from cholesterol. However, this process requires many reaction steps and the yield is less than 1 % [15]. On the other hand, the biological process has been proposed as an efficient alternative, due to the potential production of $1\alpha,25(OH)_2VD_3$ by just a two-step processes. Several researchers have reported the conversion of VD_3 to $25(OH)VD_3$ and $1\alpha,25(OH)_2VD_3$ using various microorganisms [9, 16]. The molecule $25(OH)VD_3$ was successfully produced by a VD_3-specific 25-hydroxylase, P450VD25 (CYP105A2), in a rare actinomycetes named *Pseudonocardia autotrophica* [17, 18]. According to the recent studies, VD_3–specific hydroxylases including Vdh in *P. autotrophica* and CYP105A1 in *S. griseolus* perform double hydroxylations at both positions 25 and 1α positions of VD_3 to generate $1\alpha,25(OH)_2VD_3$, yet their enzymatic kinetic values were poorly defined [9, 16]. To overcome these problems and increase the VD_3-specific bioconversion yield, new strategies, such as random mutagenesis, enzyme active site modification, and *Streptomyces* heterologous expression were also pursued [19–21].

In our previous work, 21 CYPs and their electron transfer partners (7 FD and 4 FDRs) genes were identified by whole genome sequencing of *S. benihana* and in silico analysis [22]. Furthermore, these CYPome genes were individually inactivated by the *E. coli* conjugation-based PCR-targeted gene disruption system. By using these *S. benihana* CYPome mutants, the cyclosporine A (CsA)-specific hydroxylase gene, CYP-sb21, was identified and confirmed to be responsible for the CsA regio-specific hydroxylation at the 4th *N*-methyl leucine position [22]. Here, we report that *S. benihana* is also able to perform VD_3-specific hydroxylation. By screening the *S. benihana* CYPome mutants, we prove that a single *S. benihana* CYP gene is responsible for the regio-specific hydroxylation of VD_3. In addition, we confirm the function of this newly classified VD_3-specific CYP gene through genetic complementation as well as heterologous expression in a VD_3 non-hydroxylating *Streptomyces* host.

Materials and methods

Bacterial strains and culture condition

A rare actinomycetes, *Sebekia benihana* KCTC 9610, was purchased from Korean Collection for Type Cultures (KCTC, Korea) and cultured on GSMY medium (glucose 0.7 %, yeast extract 0.45 %, malt extract 0.5 %, soluble starch 0.75 %, and calcium carbonate 0.005 %) for confirmation of VD_3 hydroxylation. It was cultured on RARE3 medium (glucose 1 %, yeast extract 0.4 %, malt extract 1 %, peptone 0.2 %, $MgCl_2{\cdot}6H_2O$ 0.359 %, glycerol 0.5 %) for conjugation experiments at 30 °C *Escherichia coli* BL21 (DE3) was grown on Luria–Bertani (LB) broth, maintained on LB agar medium at 37 °C, and supplemented with appropriate antibiotics when needed. *Streptomyces coelicolor* M145 was cultured on Tryptic Soy Broth (TSB) medium for confirmation of VD_3 hydroxylation and further cultured on YEME medium (yeast extract 0.3 %, peptone 0.5 %, malt extract 0.3 %, glucose 1 %, sucrose 34 %, and $MgCl_2{\cdot}6H_2O$ 5 mM, glycine 0.5 % separately autoclaved) for protoplast transformation at 30 °C.

HPLC analysis of VD_3-specific hydroxylation

The selected *S. benihana* strains were cultured in GSMY medium at 37 °C for 72 h, treated with VD_3 (100 mg/l) as the hydroxylation substrate, and further cultured for an additional 48 h. The crude compound was extracted from culture solution using ethyl acetate, the ethyl acetate evaporated, and the crude compound resuspended with methyl alcohol. The samples were analyzed using HPLC equipped with a photodiode array detector under the following conditions: column, YMC-Pack ODS-AM (4.6 × 300 mm) (YMC, Tokyo, Japan); UV detection, 265 nm; flow rate, 1.5 ml/min; column temperature, 40 °C and a two-buffer gradient system; ddH_2O (buffer A) and 100 % acetonitrile (buffer B). One cycle of the buffer B gradient was programmed to start at 50–100 % for 10 min followed by an elution with 100 and 50 % for 25 and 10 min, respectively. The injection volume was 20 µl.

E. coli–*S. benihana* intergeneric conjugation

A 3-day-old 10-ml *S. benihana* culture in RARE3 medium (the same media composition as described above except $MgCl_2{\cdot}6H_2O$ was added at 2 %) was diluted with 90 ml of fresh broth and grown again for 24 h at 30 °C. The culture was then centrifuged, resuspended in 10 ml of fresh medium, homogenized, and fragmented by sonication (2 cycles of 3 s on and 4 s off using an ultrasonic generator, power

36 %, Ulsso Hitech, Korea). After further growth for 20 h at 30 °C, the culture was centrifuged again, resuspended in the medium, and sonicated as before. The donor *E. coli* strain ET12567/pUZ8002, carrying the disruption construct, was prepared according to the standard *E. coli-Streptomyces* conjugation [10]. During each mating experiment, donor and recipient cells were mixed and plated on modified ISP4 medium (soluble starch 1 %, dipotassium phosphate 0.1 %, magnesium sulfate USP 0.1 %, sodium chloride 0.1 %, ammonium sulfate 0.2 %, calcium carbonate 0.2 %, ferrous sulfate 0.0001 %, manganous chloride 0.0001 %, zinc sulfate 0.0001 %, tryptone 0.15 %, yeast extract 0.05 %, agar 2 %). After 20 h of incubation at 30 °C, each plate was flooded with 1 ml of the sterile water containing hygromycin and nalidixic acid at the final concentrations of 100 and 25 μg/ml, respectively. In case of complementation, additional apramycin at the final concentration of 50 μg/ml was also included. The *S. benihana* exconjugant colonies usually appeared after 1–2 weeks of incubation.

Construction of recombinant integrative plasmid pCYPs

For the functional overexpression and complementation of the CYP-sb3 gene in *S. benihana*, a 2.4-kb DNA fragment containing the entire CYP-sb3 gene was amplified by PCR using genomic DNA from *S. benihana* as a template, along with the primers: CYP-sb3 forward primer (5′-AGATCTCCCGTCGTCATCAAG-3′) and reverse primer (5′-TCTAGAAGGGGGCCCCGGCCATAC-3′). Similarly, the CYP-sb3a gene (1.2 kb) DNA fragment was obtained using the CYP-sb3a forward primer (5′-AGATCTCCCGTCGTCATCAAG-3′) and reverse primer (5′-TCTAGAGCGTAGACGGCGTAG-3′); the CYP-sb3b gene (1.2 kb) DNA fragment was obtained using the CYP-sb3b forward primer (5′-AGATCTCCCGGCTCGGCGACATCA-3′) and reverse primer (5′-TCTAGAAGGGGGCCCCGGCCATAC-3′). Each forward and reverse primer contained *Bgl*II (AGATCT) and *Xba*I (TCTAGA) restriction enzyme sites. PCR was performed in a final volume of 20 μl containing 0.4 M of each primer, 0.25 mM of each of the four dNTPs, 1 μl of extracted DNA (400 ng/μl), 1U of Ex *Taq* polymerase (TaKaRa, Japan) in recommended reaction buffer, and 10 % dimethyl sulfoxide (DMSO). Amplifications were performed on a thermal cycler (Bio-Rad, Hercules, CA, USA) according to the following profile: 30 cycles of 60 s at 95 °C, 60 s at 58 °C, and 60 s at 75 °C. The amplified PCR product was analyzed by 1 % (w/v) agarose gel electrophoresis and purified using a DNA extraction kit (COSMO, Korea). The PCR products were then ligated into a pGEM T-easy vector (TaKaRa, Otsu, Shiga, Japan). The ligated vector was completely sequenced in order to ensure its integrity (Macrogen, Seoul, Korea). The PCR products were finally subcloned into the pSET152-derived integration plasmid containing a strong constitutive promoter, *ermE**, and a hygromycin resistance gene (Fig. 3a). The resulting plasmids were designated as pCYP-sb3, pCYP-sb3a, and pCYP-sb3b, respectively. These plasmids were introduced into *S. benihana* wild type (for overexpression), *S. benihana* ΔCYPs (for complementation) via conjugation, followed by the *att*B site integration and hygromycin selection. pCYP-sb13 (pMMBL301), which contains the CYP-sb13 (CYP506) gene, was introduced into *S. benihana* ΔCYP-sb3a as a negative control [23].

Construction of the heterologous expression plasmid pHE-CYP-sb3a

For heterologous expression of the CYP-sb3a gene in *S. coelicolor*, a 1.2-kb DNA fragment containing the entire CYP-sb3a gene was amplified using genomic DNA from *S. benihana* as a template and the CYP-sb3a Hexp forward primer (5′-CATATGAGCGACAAGCTG-3′) and reverse primer (5′-GAATTCTCGAACGGTAGTTTC-3′). The forward and reverse primers contained *Nde*I (CATATG) and *Eco*RI (GAATTC) restriction enzyme sites, respectively. PCR was performed in a final volume of 20 μl containing 0.4 M of each primer, 0.25 mM of each of the 4 dNTPs, 1 μl of extracted DNA (400 ng/μl), 1 U of Ex taq polymerase (TaKaRa, Japan) in the recommended reaction buffer, and 10 % DMSO. Amplifications were performed in a thermal cycler (Bio-Rad) according to the following profile: 30 cycles of 60 s at 95 °C, 60 s at 54 °C, and 60 s at 75 °C. The amplified PCR product was analyzed by 1 % (w/v) agarose gel electrophoresis and purified using a DNA extraction kit (COSMO, Korea). The PCR products were then ligated into a T&A cloning vector (Real Biotech Corporation, Taiwan). The ligated vector was completely sequenced in order to ensure its integrity (Macrogen). The PCR products were finally subcloned into the pHSEV-1 plasmid containing an inducible *tipA* promoter [24, 25] and the resulting plasmid was designated as pHE-CYP-sb3a. The pHE-CYP-sb3a plasmid was introduced into *S. coelicolor* via the protoplast transformation method, followed by kanamycin (kan) selection.

Heterologous expression of the *CYP-sb3a* gene in *S. coelicolor*

The recombinant plasmid pHE-CYP-sb3a was used for protoplast transformation [26]. For preparation of protoplasts, a lysozyme solution (1 mg/ml) was used treated to *S. coelicolor* which is cultured in YEME medium. The transformation was performed with pHE-CYP_{vd1} and 25 % PEG 1000 solution in P buffer (sucrose 10.3 %, K_2SO_4

0.025 %, $MgCl_2 \cdot 6H_2O$ 0.202 %, trace element solution 0.2 %). The transformed *S. coelicolor* was cultured in R2YE agar plate (sucrose 10.3 %, K_2SO_4 0.025 %, $MgCl_2 \cdot 6H_2O$ 1.012 %, glucose 1 %, casamino acids 0.01 %, agar 1.1 %. KH_2PO_4 (0.5 %) 0.1 %, $CaCl_2 \cdot 2H_2O$ (3.68 %) 0.8 %, L-proline (20 %) 0.15 %, TES buffer (5.73 %, adjusted to pH 7.2) 1 %, trace element solution 0.02 %, NaOH (1 N) 0.05 % prepared separately) and incubated at 30 °C, and kanamycin (kan) was used for the selection of recombinants.

Results and discussion

Identification of the VD_3-specific hydroxylation gene in *S. benihana*

Since *S. benihana* has been reported to introduce hydroxyl groups at specific regions of various natural products, the VD_3 bioconversion assay was also pursued to determine whether *S. benihana* is able to perform VD_3-specific hydroxylation. The *S. benihana* strain was cultured in GSMY medium at 37 °C for 72 h and VD_3 (100 mg/l) was added as the hydroxylation substrate. After an additional 48 h of culture, the crude extract was applied for HPLC analysis. Several putative VD_3 analogues were detected. Through authentic standard sample spiking and LC–MS analysis, two known VD_3 analogues were identified and confirmed as 25(OH)VD_3 (*m/z* 401.3426 [M + H]) and 1α,25$(OH)_2VD_3$ (*m/z* 417.3376 [M1-H]) (Fig. 1). These results suggest that *S. benihana* is indeed capable of performing VD_3-specific hydroxylation.

Previously, we identified the complete CYPome, including 21 CYPs and its electron transfer proteins, such as seven FD and four FDR genes [22]. The GenBank accession numbers for the *S. benihana* CYPs are KC208044 to KC208064. Each gene was then inactivated by PCR-targeted gene disruption to generate *S. benihana* CYPome mutants. A unique CYP (named CYP-sb21) was identified to be responsible for CsA-specific hydroxylation through the *S. benihana* CYPome mutant screen [22]. To identify a unique CYP gene responsible for VD_3-specific hydroxylation, the *S. benihana* CYPome mutants were similarly screened. Among the 32 mutants tested, the VD_3 conversion to both 25(OH)VD_3 and 1α,25$(OH)_2VD_3$ were only markedly decreased in the ΔCYP-sb3 and ΔCYP-sb3a

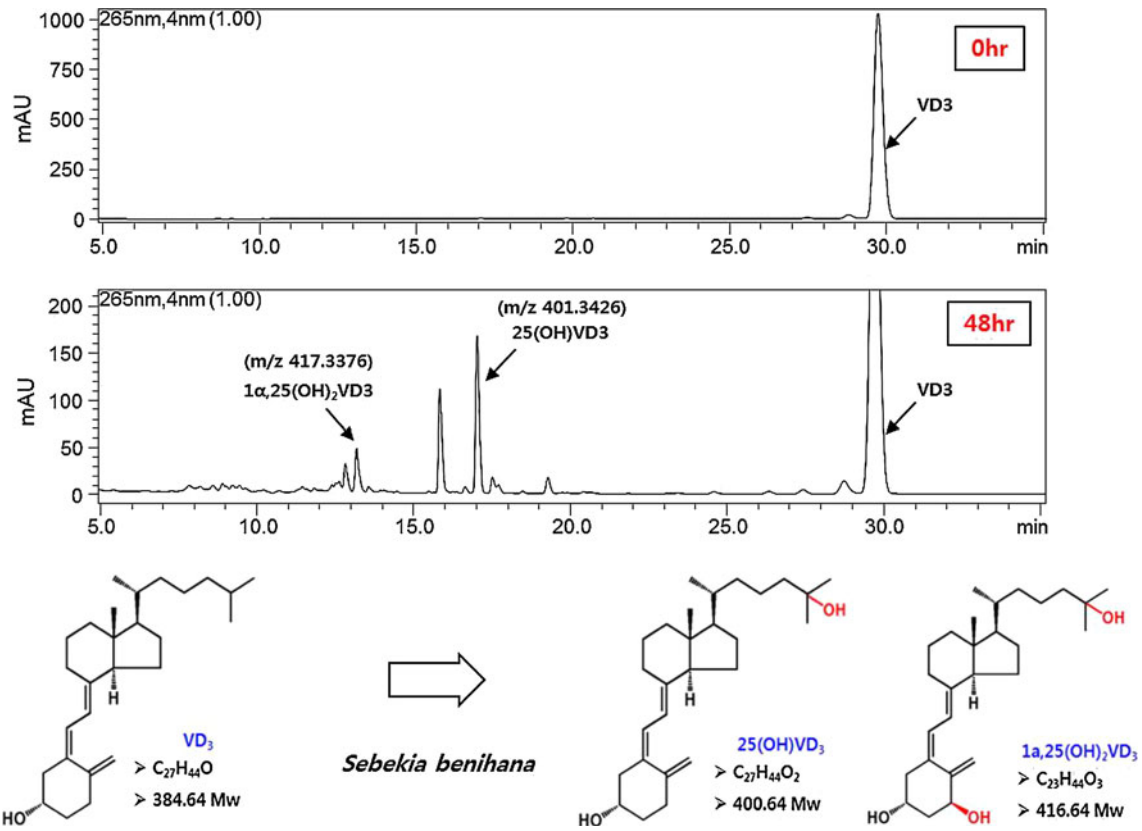

Fig. 1 Structures and HPLC profiles of VD_3 and hydroxylated VD_3 in *S. benihana*. *S. benihana* wild type was cultured in GSMY medium for 48 h and then further cultured for 48 more hours after the addition of 100 mg/l of VD_3 as the hydroxylation substrate into the culture. Samplings were conducted twice at 0 and 48 h after addition of substrate, followed by HPLC analysis

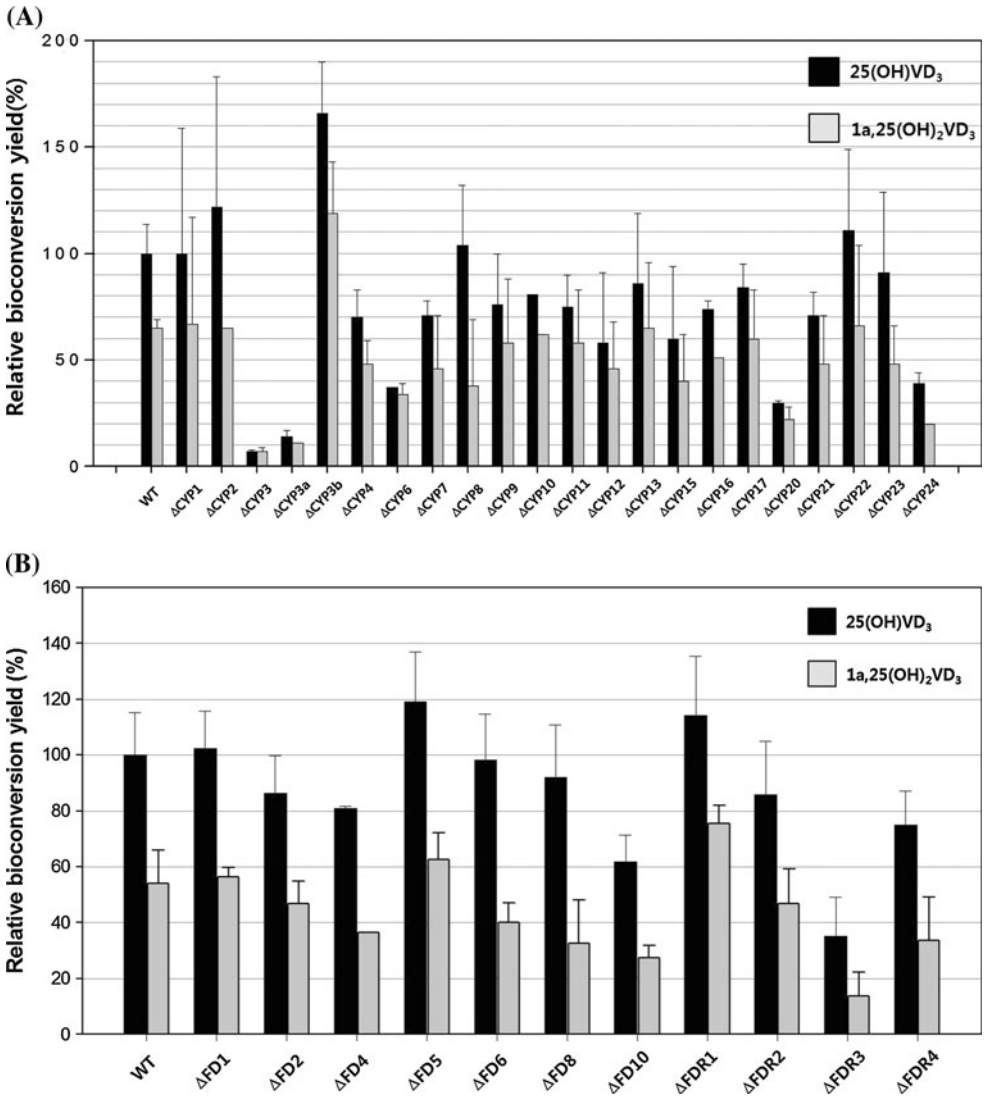

Fig. 2 Conversion yield of $25(OH)_2VD_3$ and $1\alpha,25(OH)_2VD_3$ in *S. benihana* (**a**) CYP disruptants and FD and FDR disruptants (**b**). The *vertical bars* show the standard deviation values of the mean from two separate determinations

mutant strains, even though slight reductions were also observed in some other CYP disruptants, such as ΔCYP-sb6, ΔCYP-20, and ΔCYP-24 (Fig. 2a). The ΔCYP-sb3 mutant strain contains a deletion in two translationally-coupled CYP genes, CYP-sb3a and CYP-sb3b. These results suggest that the CYP-sb3a should be mainly involved in VD_3-specific hydroxylation. Although small amounts of both $25(OH)D_3$ and $1\alpha,25(OH)_2VD_3$ remained in *S. benihana* ΔCYP-sb3a, it is likely derived from the effect of other non-specific CYPs. On the other hand, most *S. benihana* ΔFD-sb and ΔFDR-sb mutants still maintained VD_3 hydroxylation activities (Fig. 2b), implying that no single FD or FDR is responsible for VD_3-specific hydroxylation. Since FD and FDR, involved in electron transfer from NAD(P)H to the heme–iron of CYP, exist in lower numbers in most actinomycetes species, *S. benihana* VD_3-specific CYP-sb3a is believed to have no specific preference toward FD or FDR in VD_3-specific hydroxylation [27–29].

Complementation and characterization of the *CYP-sb3a* gene in *S. benihana*

Gene complementation of CYP-sb3a in *S. benihana* ΔCYP-sb3a was performed to further confirm the role of CYP-sb3a in VD_3-specific hydroxylation. The coding sequence of CYP-sb3a was cloned into a chromosome integrative plasmid including the constitutive *ermE** promoter. The pCYP-sb3a was introduced into the genome of *S. benihana* ΔCYP-sb3a by conjugation, and the genomic DNA of *S. benihana* ΔCYP-sb3a/pCYP-sb3a was confirmed by PCR amplification. The HPLC results confirmed that the VD_3 hydroxylation to $25(OH)VD_3$ and $1\alpha,25(OH)_2VD_3$ were significantly restored in *S. benihana*

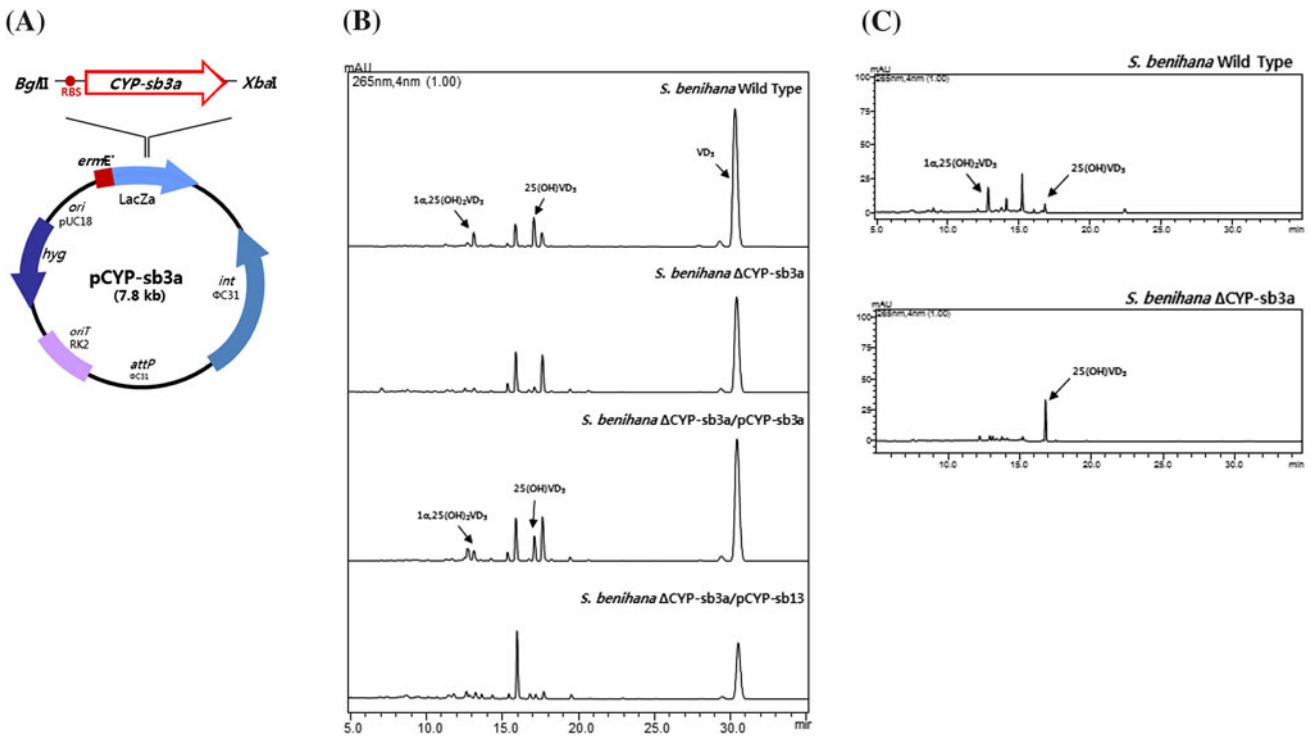

Fig. 3 Genetic complementation of *S. benihana* ΔCYP-sb3a. **a** Construction of the recombinant plasmid pCYP-sb3a. **b** HPLC profiles of VD_3 bioconversion in the *S. benihana* wild type, *S. benihana* ΔCYP-sb3a, *S. benihana* ΔCYP-sb3a/pCYP-sb3a, and *S. benihana* ΔCYP-sb3a/pCYP-sb13 strains. **c** Bioconversion of $25(OH)_2VD_3$ to $1\alpha,25(OH)_2VD_3$ in the *S. benihana* wild type and *S. benihana* ΔCYP-sb3a

ΔCYP-sb3a/pCYP-sb3a (Fig. 3a). On the other hand, overexpression of a CYP-sb13 (as a negative control) in *S. benihana* ΔCYP-sb3a failed to restore the production of both $25(OH)D_3$ and $1\alpha,25(OH)_2VD_3$ (Fig. 3b). When $25(OH)VD_3$ was added as a hydroxylation substrate in the *S. benihana* culture, $25(OH)VD_3$ was further converted to $1\alpha,25(OH)_2VD_3$ in the *S. benihana* wild-type but not in the ΔCYP-sb3a mutant (Fig. 3c). These results imply that VD_3 hydroxylation occurs at position 25 first, followed by the second hydroxylation at position 1α. $25(OH)VD_3$ was not further converted to $1\alpha,25(OH)_2VD_3$ in the ΔCYP-sb3a mutant, implying that *S. benihana* CYP-sb3a is responsible for VD_3 hydroxylation at both positions 25 and 1α.

We analyzed the CYP-specific motif in CYP-sb3a by comparing its amino acids with previously reported VD_3-specific CYPs (Fig. 4a). There are typically three CYP-conserved motifs present in CYP amino acid sequences. The GXXXCXG motif is a region of the cysteine heme–iron ligand, the EXXR motif in the K-helix region is involved in the interaction between oxidation–reduction partners and the GXXT motif is the oxygen-binding site [30]. The sequence homology and phylogenetic results shown in Fig. 4b exhibited higher amino acid similarity with the CYP107 family VD_3 hydroxylases, Vdh (41 % identity) in *P. autotrophica* than the CYP105 family VD_3 hydroxylases, CYP105A1 (32 % identity) in *S. griseolus* [9]. These results suggest that the *S. benihana* CYP-sb3a, which performs double hydroxylations at both positions 25 and 1α positions of VD_3, belongs to the bacterial CYP107 family like the *P. autotrophica* Vdh.

Functional expression of the *CYP-sb3a* gene in a heterologous host

To confirm the in vivo function of CYP-sb3a in a heterologous host, we used *S. coelicolor* M145 as an expression host, due to its easy genetic manipulation. More importantly, *S. coelicolor* does not carry out VD_3-specific hydroxylation. The coding sequence of the *CYP-sb3a* gene was cloned into pHSEV-1, which contains a thiostrepton-induced *tipA* promoter (pHE-CYP-sb3a, Fig. 5a), and this recombinant plasmid was introduced into *S. coelicolor* M145 through polyethylene glycol (PEG)-assisted protoplast transformation followed by kanamycin selection. $25(OH)VD_3$ was clearly detected in the *S. coelicolor* M145/pHE-CYP-sb3a strain, while no such hydroxylation was observed in the *S. coelicolor* M145 wild-type strain containing the vector alone (Fig. 5b). These results confirm that CYP-sb3a plays a

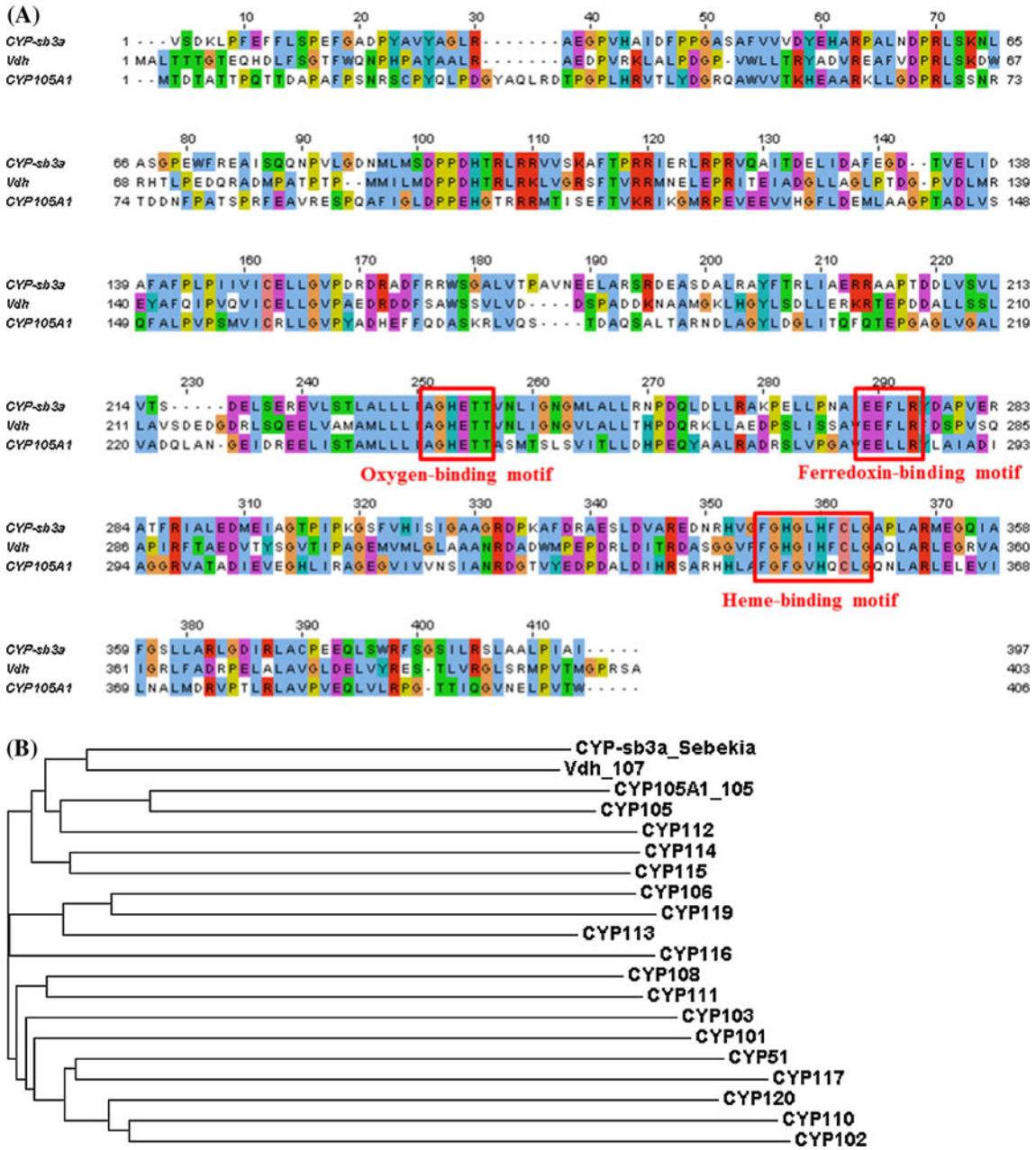

Fig. 4 **a** Sequence alignment of CYP-sb3a, Vdh from *Pseudonocardia autotrophica* and CYP105A1 from *Streptomyces griseolus*. *Boxes* represent conserved amino acid residues that constitute the CYP-specific motif. **b** Phylogenetic tree of CYP-sb3a and other CYP families. CYP-sb3a (*S. benihana*), Vdh (*Pseudonocardia autotrophica*), CYP105A1 (*S. griseolus*). CYP51 (*Nocardia farcinica*), CYP101 (*Novosphingobium aromaticivorans*), CYP102 (*Bacillus anthracis*), CYP103 (*Agrobacterium tumefaciens*), CYP105 (*Saccharopolyspora erythraea*), CYP106 (*B. anthracis*), CYP110 (*Nostoc* sp.); CYP111 (*N. aromaticivorans*), CYP112 (*Sinorhizobium fredii*), CYP113 (*S. erythraea*), CYP114 (*M. loti*), CYP115 (*M. loti*), CYP116 (*S. erythraea*), CYP117 (*S. fredii*); CYP119 (*Sulfolobus tokodaii*), CYP120 (*Trichodesmium erythraeum*)

major role in VD_3-specific hydroxylation, even in a heterologous host. Interestingly, however, no clear $1\alpha,25(OH)_2VD_3$ peak was detected in the *S. coelicolor* M145/pHE-CYP-sb3a strain, implying that the efficiency of the second hydroxylation at position 1α of $25(OH)VD_3$ is not as good as the first hydroxylation at position 25 of VD_3 in a heterologous host system. We cannot rule out the possibility that some additional factors present in *S. benihana*, besides CYP-sb3a, may be required for the second hydroxylation in *S. coelicolor*. In conclusion, this is the first report that *S. benihana* CYP-sb3a, a new member of the bacterial CYP107 family, is the key

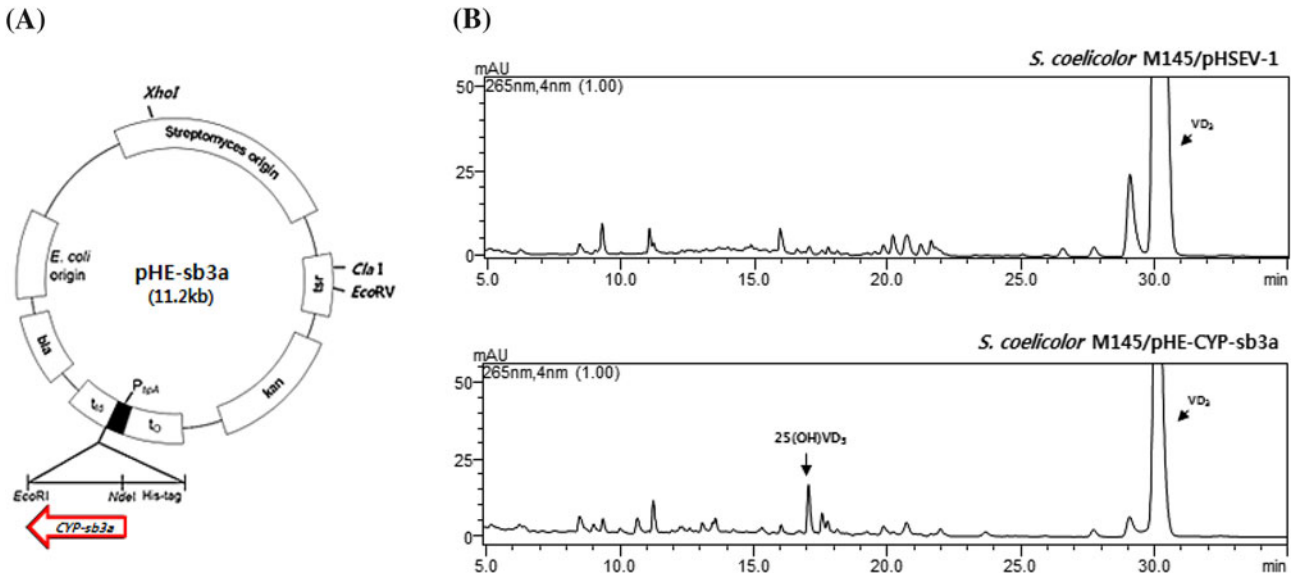

Fig. 5 **a** Map of the pHE-CYP-sb3a plasmid, a pHSEV-1 derivative [24, 25]. **b** The HPLC profiles of VD_3 metabolites with heterologous expression of CYP-sb3a in *S. coelicolor* M145/pHE-CYP-sb3a

cytochrome P450 hydroxylase involved in regio-specific VD_3 hydroxylation.

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