Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 361 (2007) 196–201
BBRC
www.elsevier.com/locate/ybbrc

SanM catalyzes the formation of 4-pyridyl-2-oxo-4-hydroxyisovalerate in nikkomycin biosynthesis by interacting with SanN

Hongbo Ling [a,b], Guojun Wang [a,1], Yuqing Tian [a], Gang Liu [a], Huarong Tan [a,*]

[a] State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
[b] Graduate School of Chinese Academy of Sciences, Beijing 100039, China

Received 1 July 2007
Available online 16 July 2007

Abstract

Nikkomycins are peptidyl nucleoside antibiotics with potent activities against phytopathogenic and human pathogenic fungi. The *sanM* and *sanN* genes are required for the nikkomycin biosynthesis of *Streptomyces ansochromogenes*. In the present study, interaction between SanM and SanN was identified by yeast two-hybrid and co-immunoprecipitation assays. Moreover, SanM and SanN were heterologously expressed and purified. Further biochemical assay demonstrated that the SanM–SanN interaction is essential for SanM aldolase activity but not for SanN dehydrogenase activity. SanM converts piconaldehyde and 2-oxobutyrate to 4-pyridyl-2-oxo-4-hydroxyisovalerate in nikkomycin biosynthesis by interacting with SanN. Steady state kinetics analysis revealed that K_m and k_{cat}/K_m of SanM are 123.2 μM and 11.4 mM^{-1} s^{-1} for picolinaldehyde, while 335.6 μM and 4.0 mM^{-1} s^{-1} for 2-oxobutyrate, respectively. However, SanN as a dehydrogenase is independent of SanM.

Keywords: SanM; SanN; Interaction; Aldolase; Dehydrogenase; Nikkomycin biosynthesis

Nikkomycins are a group of peptidyl nucleoside antibiotics produced by *Streptomyces tendae* Tü901 [1] and *Streptomyces ansochromogenes* 7100 [2]. Due to their structural similarity to UDP-*N*-acetylglucosamine, nikkomycins act as a competitive inhibitor of chitin synthetase and show potent activities against phytopathogenic and human pathogenic fungi (e.g., *Alternaria longipes*, *Aspergillus fumigatus, Candida albicans*, etc.). Furthermore, nikkomycins display low toxicity to mammals and are easily degraded in natural environment, they can be used as ideal fungicidal agents in agriculture and also as antifungal agents in human therapy [3–5].

Nikkomycins contain a peptidyl moiety and a nucleoside moiety. The main compounds nikkomycin X and Z are shown in Fig. 1A. Their peptidyl moieties are hydroxypyridyl-homothreonine (HPHT), while nucleoside moieties consist of an aminohexuronic acid with *N*-glycosidically bond 4-formyl-4-imidazoline-2-one or uracil. The two moieties of nikkomycins are assumed to be formed in separate ways and then combined with peptide bonds [6,7]. HPHT biosynthesis is proposed to involve more than 11 enzymatic reactions, and four of them have been clearly studied in *S. tendae* or *S. ansochromognenes* up to now [8–11] (Fig. 1B). Moreover, 4-pyridyl-2-oxo-4-hydroxyisovalerate (POHIV) had been isolated from culture filtrates of *S. tendae* and proved to be a precursor of HPHT [13]. In earlier study, *nikA* and *nikB* (respective GenBank Accession Nos. CAB46531 and CAB46532) in *S. tandae* were deduced to participate in the POHIV formation [12]. However, no further biochemical datum was provided and the exact functions of *nikA* and *nikB* genes in nikkomycin biosynthesis still remain unclear.

We previously cloned the entire gene cluster for nikkomycin biosynthesis in *S. ansochromogenes,* which is at least composed of three transcriptional units with 25 genes [14].

* Corresponding author. Fax: +86 10 64807461.
E-mail address: tanhr@sun.im.ac.cn (H. Tan).
[1] Present address: Microbial Function Laboratory, National Food Research Institute, Tsukuba 305-8642, Japan.

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.07.016

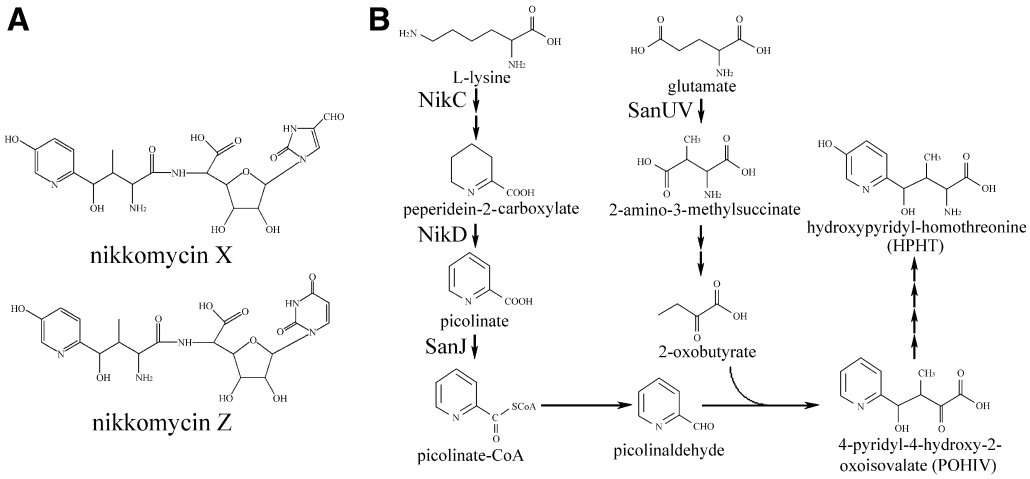

Fig. 1. Chemical structures of nikkomycin X and Z and proposedly biosynthetic pathway of hydroxypyridyl-homothreonine (HPHT). (A) Chemical structures of nikkomycin X and Z. (B) Proposedly biosynthetic pathway of HPHT. Both SanJ as a picolinate–CoA ligase and SanUV as a glutamate mutase involved in the nikkomycin biosynthesis of *S. ansochromogenes* were characterized in our previous work [10,11]. Partial pathway is based on that proposed by Bruntner et al. [12].

Among them, the *sanN* and *sanM* are respective counterparts of *nikA* and *nikB*, and play essential roles in nikkomycin biosynthesis of *S. ansochromogenes*. In this paper, we report that SanM converts picolinaldehyde and 2-oxobutyrate to POHIV by interacting with SanN, whereas SanN as a dehydrogenase is independent of SanM.

Materials and methods

Strains, plasmids, and media. *S. ansochromogenes* 7100, a natural nikkomycin producer, was isolated from the soil of Northeastern China [2]. *Saccharomyces cerevisiae* AH109 and Y187 and corresponding plasmids, pGBKT7, pACT2, etc. (Clontech), were used to perform yeast two-hybrid assay. *Escherichia coli* BL21 (DE3) (Novagen) and *S. lividans* TK24 [15], and plasmids pET23b (Novagen) and pIJ8600 [15] were used as host strains and vectors for heterologous expression of proteins, respectively. Methylation-deficient *E. coli* strain ET12567/pUZ8002 (*dam dcm hsdS cat tet tra* RP4 kan^R) was used for conjugal transfer of DNA from *E. coli* to *Streptomyces* [16,17].

All media for *Streptomyces* growth were prepared as previously described [12,15]. Other media for *E. coli* or yeast growth were prepared according to standard protocol [18]. When necessary, antibiotics were used at the following concentrations: ampicillin (Amp), 100 μg/ml in LB; apramycin (Apr), 10 μg/ml in YEME or MM, 30 μg/ml in MS or 50 μg/ml in LB; kanamycin (Kan), 10 μg/ml in MM, 50 μg/ml in LB; tetracycline and chloramphenicol, 10 μg/ml and 25 μg/ml in LB, respectively.

Construction of plasmids. Plasmids pGBKT7-M and pACT2-N were used to express BD-SanM and AD-SanN in yeast two-hybrid assay. To construct pGBKT7-M, the encoding region of *sanM* was amplified from pNL5400 with primer P1, 5′-GGAATTCCATATGAGCCAGGAAGCGG-3′ (NdeI sites underlined), and P2, TCAGCTGCAGCCCGTTCACGGTCAGCATG (PstI sites underlined). The resulting product was digested with NdeI and PstI and cloned into the same sites of pGBKT7 to generate pGBKT7-M. Similarly, *sanN* was amplified using primers P3, 5′-CCCCATGGAGATGCAAGCAACTGGACGG-3′ (NcoI sites underlined), and P4, 5′-TTACTCGAGTGCCGTCTCCCCTGCC-3′ (XhoI sites underlined), digested with NcoI and XhoI, and ligated into the same sites of pACT2 to give pACT2-N. To generate pET23b-M and pET23b-N for expression of SanM and SanN in *E. coli*, *sanM* was amplified from pNL5400 with primers P1 and P5, 5′-TTACTCGAGGCATGAGCCGGT-3′ (XhoI sites underlined), and *sanN* was amplified with primers P6, 5′-GGAATTCCATATGCAAGCAACTGGACGA-3′ (NdeI sites underlined), and P4. The amplified *sanM* and *sanN* were digested with NdeI and XhoI, and inserted into the same sites of pET23b. To construct pIJ8600-M and pIJ8600-N for expression of SanM and SanN in *S. lividans, sanM* and *sanN* genes fused with his-tag were amplified from pET23b-M and pET23b-N with primers P7, 5′-CGATCCCGCGAAATTAATAC-3′, and P8, 5′-CCGGATCCAAAAAACCCCTCAAGACC-3′ (BamHI sites underlined), respectively. Then, the PCR products were digested with NdeI and BamHI, and ligated with the NdeI–BamHI digested pIJ8600.

Preparation of antibody and Western blot analysis. The pET23b-M and pET23b-N were transformed into *E. coli* BL21 (DE3) for expressions of His_6-SanM and His_6-SanN, respectively. Both SanM and SanN were expressed in *E. coli* as inclusion bodies and were further purified. Approximately 2 mg of purified His_6-SanM or His_6-SanN mixed with Freund's complete adjuvant was injected into two healthy mice for three times (once per week), respectively. Antisera were collected from the eyes of the immune mice at day 30 and used as the polyclonal antibodies against SanM or SanN.

For Western blot analysis, the supernatants of cell lysates or adsorbed beads (immunoprecipitates) were immediately boiled for 10 min, subjected to 10% SDS–PAGE, and electro-blotted to a nitrocellulose membrane. Non-specific binding was blocked with 5% fat-free milk in PBS overnight at 4 °C and rinsed twice with 0.1% Tween 20/PBS. Then the membrane was incubated with anti-SanN or anti-SanM antibody at room temperature for 1 h, followed by HRP-labeled anti-mouse IgG antibody for 1 h. Reaction product was visualized with enhanced chemiluminescence according to the kit instructions (Amersham Pharmacia Biotech) and documented on an X-ray film.

Yeast two-hybrid assay. The yeast two-hybrid assay was performed as described in the MATCHMAKER GAL4 Two-Hybrid System 3 (Clontech). Yeast strain AH109 was transformed with pGBKT7-M or control plasmids (pGBKT7-53 and pGBKT7-Lam), and yeast strain Y187 was transformed with pACT2-N or control plasmid (pGADT7-T) according to the small-scale LiAc yeast transformation procedure. Yeast mating method was used to identify the SanM–SanN interaction. In brief, the recombinant yeast strain, AH109/pGBKT7-M, was mated with strain Y187/pACT2-N. Then, the co-transformants were selected on SD/-Leu/-Trp medium at 30 °C for 5 d and further analyzed for the β-galactosidase activity. The positive control was prepared by mating of AH109/pGBKT7-53 with Y187/pGADT7-T. Negative controls were prepared by mating of AH109/pGBKT7-Lam with Y187/pACT2-N and by mating of AH109/pGBKT7-M with Y187.

Co-immunoprecipitation assay. For immunoprecipitation, Sepharose 4B beads conjugated to protein G (Amersham Pharmacia Biotech) were incubated with anti-SanN or anti-SanM antibody for 1 h at 4 °C and were washed three times with HEPES buffer (pH 7.5). Then the beads were incubated with the cell extracts of *S. ansochromogenes* 7100 at 4 °C, washed with HEPES buffer, and analyzed by immunoblotting with anti-SanM or anti-SanN antibody.

Expression of SanM and SanN in S. lividans TK24. The pIJ8600-M and pIJ8600-N were introduced into *S. lividans* TK24 by conjugation as described previously [10]. The resulting recombinant strains were grown in YEME at 28 °C for 36 h, further induced with 5 μg/ml thiostrepton for another 12 h. Then, cultures were collected, washed, and sonicated until completely lysed. SanM and SanN proteins were purified from the supernatants by Ni^{2+} affinity chromatography and subjected to SDS–PAGE analysis as described previously [11].

Enzyme activity assays. All enzyme activity assays were performed at 28 °C. To determine aldolase activity, the standard reaction mixture (1 ml) contained 50 mM HEPES buffer (pH 8.0), 1 mM $MnCl_2$, 1 mM dithiothreitol, 2 mM picolinaldehyde, 4 mM 2-oxobutyrate, and 50 nM purifed enzymes. After incubation for 3 h, aldolase activity was measured by analyzing the reaction products with reverse-phase HPLC followed by LC/ESI-MS. For HPLC analysis, 10 μl of reaction mixtures without dilution was injected into column (Zorbax Eclipse XDB-C18, 4.6 mm × 250 mm, 5 μm; Agilent) pre-equilibrated with 0.5‰ trifluoroacetic acid (v/v) and eluted with the same solution at a flow rate of 0.8 ml/min at 30 °C. The elution was monitored with UV/vis diode array at 260 nm. LC/ESI-MS was further used to determine molecular weight as previously described [10]. The standard reaction mixture (1 ml) for detecting dehydrogenase activity contained 50 mM Tris–Cl (pH 7.5), 1 mM dithiothreitol, 2 mM benzoate–CoA, 600 μM NADH, and 50 nM purifed enzymes. The reaction was determined by monitoring the oxidation of NADH (OD_{340}) in enzyme coupling assay.

For assays at different pH value, the reactions were performed with the 50 mM following buffers and pH values (in parentheses): potassium phosphate (6.0–8.0), HEPES–NaOH (7.0–8.0), Tris–HCl (7.5–9.0), and glycine–NaOH (9.0–10.0). Kinetic parameters for aldolase enzyme were determined by three separate measurements at each point. The kinetic results were fitted for Michaelis–Menten equation and calculated using a Lineweaver–Burk plot.

Nucleotide sequence accession number. The nucleotide sequence of *sanN* and *sanM* was deposited in GenBank under Accession Nos. AY188795 and AF469949, respectively.

Results

Sequence analysis of sanN and sanM

SanN protein shows about 46% end-to-end identity to putative acetaldehyde dehydrogenase (ADA, GenBank Accession No. NP639626) and semialdehyde dehydrogenase (GenBank Accession No. ZP_01534271), containing a semialdehyde dehydrogenase domain and AcetDehyd-dimer at its N-terminus and C-terminus, respectively. SanM protein shows about 68% identity to a putative 4-hydroxy-2-oxovalerate aldolase (HOA, GenBank Accession No. NP_639628) and a putative pyruvate carboxyltransferase (GenBank Accession No. ZP_01534272), containing a pyruvate carboxyltransferase domain at the N-terminus and a DmpG communication domain at the C-terminus.

Moreover, *sanNM* gene pairs are highly conserved with HOA/ADA-encoding gene pairs in degradation pathway of aromatic compounds, not only in the base sequences but also in the order of genes. In all these gene pairs, ADA-encoding genes are located upstream the HOA-encoding genes [19–23].

SanM is associated with SanN

Sequence analysis demonstrated that there is possible co-evolution between *sanNM* gene pairs and HOA/ADA-encoding gene pairs, indicating the possibility of the physical association of SanM and SanN. Furthermore, SanM protein coantains a DmpG communication-like domain, which is thought to promote heterodimerization in DmpFG [24]. Therefore, we speculate that SanM may interact with SanN.

Yeast two-hybrid assay was used to test the interaction between SanM and SanN with yeast mating. The mating experiment showed that the co-transformants, resulting from mating of AH109/pGBKT7-M with Y187/pACT2-N, could grow on SD/-Leu/-Trp medium; and the interaction of AD-SanN with BD-SanM initiated the expression of β-galactosidase, which hydrolyzed substrate X-Gal and released blue products, the same as co-transformants resulting from mating of AH109/pGBKT7-53 with Y187/pACT2-T (positive control). As a negative control, co-transformants resulting from mating of Y187/pACT2N with AH109/pGBKT7-Lam could grow on SD/-Leu/-Trp medium, but it did not display β-galactosidase activity. As the other negative control, co-transformants resulting from mating of AH109/pGBKT7-M with Y187 could not grow on SD/-Leu/-Trp medium (Fig. 2A).

Co-immunoprecipitation assay was further carried out to identify SanM–SanN interaction. Results showed that anti-SanM antibody could co-immunoprecipitate SanM with SanN in *S. ansochromogenes* 7100. Conversely, anti-SanN antibody could co-immunoprecipitate SanN with SanM too. As control, normal mouse IgG failed to pull down any detectable SanN or SanM (Fig. 2B).

These results demonstrated that SanM could interact with SanN, suggesting that both of the proteins may function as SanMN complex in vivo.

Expression and purification of SanM and SanN

Since both SanM and SanN proteins were insoluble and inactive when expressed in *E. coli* under variously optimized conditions, we used *S. lividans* TK24 instead of *E. coli* as host strain for heterologous expression of SanM and SanN by the induction of 5 μg/ml thiostrepton under the control of the *tipA*p. The recombinant SanM and SanN were soluble and purified by Ni^{2+} affinity chromatography, and showed on SDS–PAGE at the expected size of about 38.2 kD and 32.5 kD, respectively (data not shown).

SanM converts picolinaldehyde and 2-oxobutyrate to POHIV by interacting with SanN

As SanM contains a pyruvate carboxyltransferase domain, we inferred that SanM catalyzes the formation

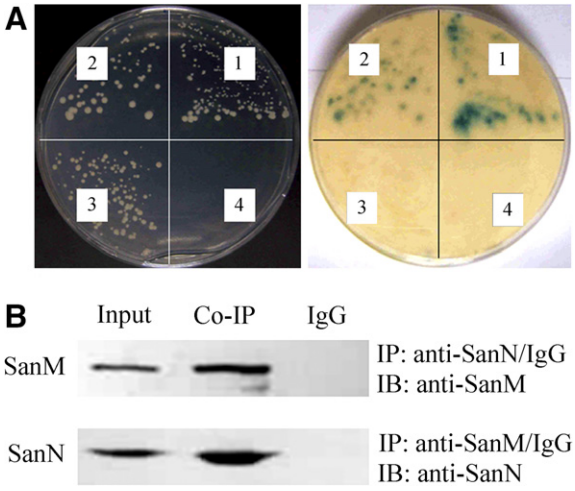

Fig. 2. Identification of SanM–SanN interaction by yeast two-hybrid and co-immunoprecipitation assays. (A) Yeast two-hybrid assay. The selective growth on SD/-Leu/-Trp plate (left panel) and analysis of β-galactosidase activity of co-transformants (right panel). Co-transformants were, respectively, obtained from 1, mating of AH109/pGBKT7-53 with Y187/pGADT7-T (positive control); 2, mating of Y187/pACT2-N with AH109/pGBKT7-M; 3, mating of AH109/pGBKT7-Lam with Y187/pACT2-N (a negative control); and 4, mating of AH109/pGBKT7-M with Y187 (the other negative control). (B) Co-immunoprecipitation. Sepharose 4B beads conjugated to protein G (Amersham Pharmacia Biotech) were incubated with anti-SanN or anti-SanM antibody (normal mouse IgG was used as control) for 1 h at 4 °C and were washed three times with HEPES buffer (pH 7.5). Then, the beads were incubated with the supernatant of cell lysates of *S. ansochromogenes* 7100 overnight at 4 °C for immunoprecipitating (IP) SanN or SanM, washed with HEPES buffer, and analyzed by immunoblotting (IB) with anti-SanM or anti-SanN antibody. Cell extract from *S. ansochromogenes* 7100 served as input. The precipitation with anti-SanM antibody could pull down SanM with SanN and vice versa. As control, normal mouse IgG failed to pull down any detectable SanN or SanM.

of POHIV. The reaction product mixtures catalyzed by purified individual SanM and SanN as well as SanM/SanN mixture were subjected to reverse-phase HPLC analysis. In comparison with the reaction product catalyzed by individual SanM or SanN, a new prominent peak at 5.3 min was observed in that by SanM/SanN mixture. Further LC/ESI-MS analysis for the new compound revealed a single $[M+H]^+$ peak at *m/z* 210.2, which is in accordance with the calculated value for POHIV (Fig. 3). Results indicated that SanM converts picolinaldehyde and 2-oxobutyrate to POHIV by interacting with SanN, whereas, individual SanM or SanN failed to catalyze the reaction and no corresponding product was detected under the same condition. These further confirmed that SanM and SanN may form a SanMN complex to act its function in nikkomycin biosynthesis.

Moreover, activity of SanM as an aldolase varied with the different molar ratio of SanN to SanM and approached saturation above 1:1, indicating SanM and SanN may form SanMN complex at a molar ratio of 1:1. The optimal pH value for SanM aldolase is 8.0. Mn^{2+} (1 mM) stimulated the aldolase activity about fourfold, but Zn^{2+}, Mg^{2+}, and Ca^{2+} had no effect.

Initial velocities were determined in the standard assay mixture at pH 8.0 for subsequent kinetics analysis. In both cases, regular saturation curves of the activity versus picolinaldehyde and 2-oxobutyrate concentrations were observed (Fig. 4). The K_m value of SanM toward picolinaldehyde and 2-oxobutyrate in aldol condensation reaction was calculated from the Lineweaver–Burk plot to be 123.2 μM and 335.6 μM. The calculated k_{cat}/K_m of SanM is 11.4 $mM^{-1}\,s^{-1}$ and 4.0 $mM^{-1}\,s^{-1}$ for picolinaldehyde and 2-oxobutyrate, respectively.

SanN shows dehydrogenase activity

SanN shares significant similarities to ADA and semialdehyde dehydrogenase. On this basis, we postulated that SanN may catalyze the conversion of picolinate–CoA to picolinaldehyde in nikkomycin biosynthetic pathway (Fig. 1B). Here, benzoate–CoA, the structural analog of picolinate–CoA, was used as the substrate to investigate the dehydrogenase activity of individual SanN and SanM as well as SanM/SanN mixture. Interestingly, individual SanN shows the dehydrogenase activity and the addition of SanM has no effect on it. The optimal pH of SanN dehydrogenase is 7.5. No stimulation of activity was observed when Mn^{2+}, Mg^{2+}, Ca^{2+}, or Zn^{2+} (1 mM) was added to Tris-buffered assay mixtures.

Discussion

The *sanN* and *sanM* genes are required for nikkomycin biosynthesis of *S. ansochromogenes* 7100. Our results revealed that SanM acts as a POHIV aldolase, converting picolinaldehyde and 2-oxobutyrate to POHIV by interacting with SanN, and SanM and SanN may form a bifunctional aldolase/dehydrogenase complex. Interestingly, the interaction of SanM with SanN is essential for the SanM aldolase activity but not for SanN dehydrogenase activity.

It was indicated that the aldolase activity of SanM may be activated by the association of SanN. Recently, the expression of the MhpE and MhpF, a pair of HOA and ADA from *E. coli* similar to SanM and SanN, was studied in detail. The MhpE aldolase was soluble only when soluble MhpF was available, suggesting that the soluble MhpF functions as a chaperone protein for folding of MhpE [25]. We had also tried to express the SanM and SanN protein in *E. coli*, but both SanM and SanN are insoluble in *E. coli* under various conditions. However, both SanM and SanN are soluble when heterologously expressed in *S. lividans* TK24. Perhaps there exists a different expression mechanism in *S. lividans* TK24 and in *E. coli* for folding SanM and SanN. Nevertheless, purified SanM failed to exhibit aldolase activity until the addition of SanN, indicating that reassembly of SanMN complex may achieve the aldolase activity. These demonstrated that SanN not only acts as a chaperone protein for folding of SanM, like MhpF for MbpE, but also activates the SanM to function as aldolase.

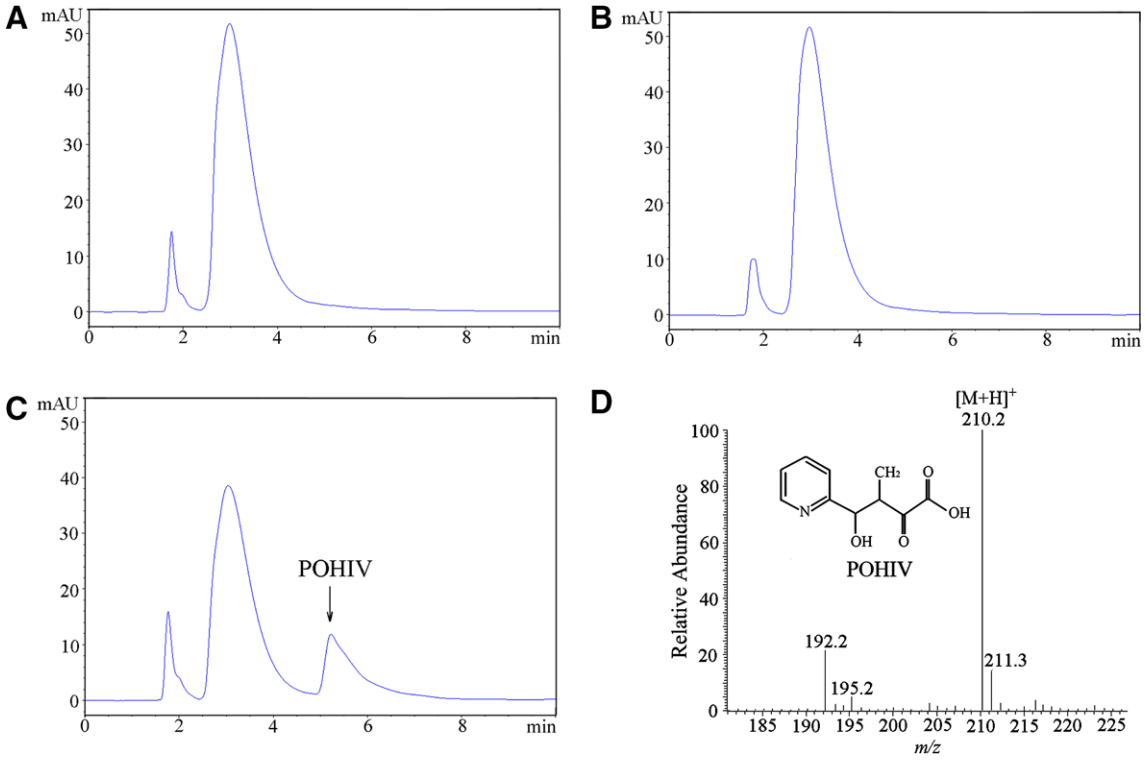

Fig. 3. Conversion of picolinaldehyde and 2-oxobutyrate to POHIV catalyzed by SanM/SanN mixture. The reaction products catalyzed by individual SanM (A) and SanN (B) as well as SanM/SanN mixture (C) were subjected to HPLC analysis as described in Materials and methods. Further LC/ESI-MS for the novel compound presented in reaction products catalyzed by SanM/SanN mixture revealed a single $[M+H]^+$ peak at *m/z* 210.2, which is in accordance with the calculated value for POHIV (D).

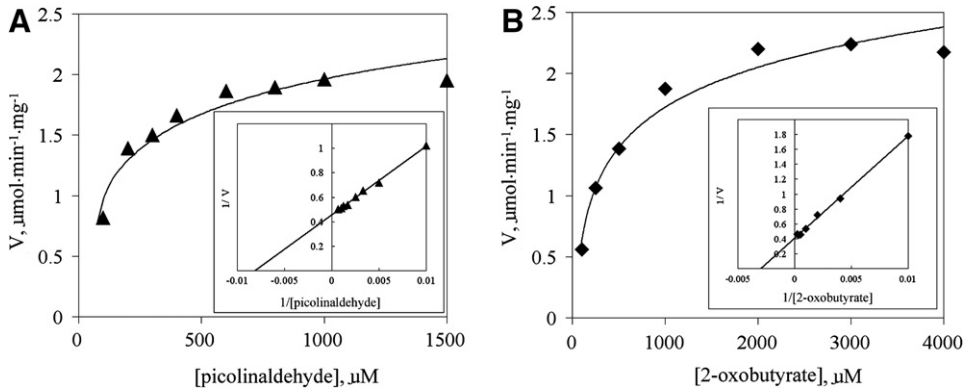

Fig. 4. Effect of substrate concentration on SanM aldolase activity. Each inset shows a Lineweaver–Burk plot of initial velocity versus various fixed substrate concentrations. In panel (A) 2-oxobutyrate concentration was kept constant at 2000 μM, the picolinaldehyde concentration varied as figure indicates. In panel (B) picolinaldehyde concentration was kept constant at 2000 μM, and 2-oxobutyrate concentration varied as figure indicates.

Another possible explanation is that SanN might be a cooperative partner to SanM, and both of them form the internal channel by protein–protein interactions to facilitate the aldol reaction, as the DmpGF complex, a bifunctional HOA/ADA similar to SanM and SanN. In DmpG, an acetyl group is removed from 4-hydroxy-2-ketovalerate, and transferred by means of a long channel to CoA on the DmpF domain, followed by a CoA-dependent acylating aldehyde dehydrogenation reaction [24]. More intensive investigations on the physical properties and catalytic mechanism of SanMN are needed to further elucidate molecular event in the aldol condensation.

Aldolase/dehydrogenase (HOA/ADA)-encoding gene pairs were extensively found in degradation pathway of aromatic compounds [21,22,26,27]. However, to our knowledge, it is the first time a bifunctional aldolase/dehydrogenase complex in the biosynthetic pathway of antibiotics has been reported. These data would be of benefit to elucidation of the nikkomycin biosynthesis and combinatorial synthesis of novel antibiotic derivatives.

Acknowledgments

This work was supported by grants from the Ministry of Science and Technology of China (Grant Nos. 2003CB114205 and 2006AA02Z206) and Chinese Academy of Sciences (Grant No. KSCX2-YW-N-027). We thank Prof. Keith Chater (John Innes Center, Norwich, UK) for providing strain *E. coli* ET12567/pUZ8002, plasmid pIJ8600 and for helpful discussion during this work.

References

[1] G.U. Brillinger, Metabolic products of microorganisms. 181. Chitin synthase from fungi, a test model for substances with insecticidal properties, Arch. Microbiol. 121 (1979) 71–74.
[2] W. Chen, H. Zeng, H. Tan, Cloning, sequencing, and function of *sanF*: a gene involved in nikkomycin biosynthesis of *Streptomyces ansochromogenes*, Curr. Microbiol. 41 (2000) 312–316.
[3] H.P. Fiedler, R. Kurth, J. Langharig, J. Delzer, H. Zahner, Nikkomycins: microbial inhibitors of chitin synthetase, J. Chem. Technol. Biotechnol. 32 (1982) 271–280.
[4] R.F. Hector, B.L. Zimmer, D. Pappagianis, Evaluation of nikkomycins X and Z in murine models of coccidioidomycosis, histoplasmosis, and blastomycosis, Antimicrob. Agents Chemother. 34 (1990) 587–593.
[5] L.T. Ganesan, E.K. Manavathu, J.L. Cutright, G.J. Alangaden, P.H. Chandrasekar, In-vitro activity of nikkomycin Z alone and in combination with polyenes, triazoles or echinocandins against *Aspergillus fumigatus*, Clin. Microbiol. Infect. 10 (2004) 961–966.
[6] P. Engel, A.H. Ullah, Mutation affecting peptide bond formation in nikkomycin biosynthesis, Biochem. Biophys. Res. Commun. 156 (1988) 695–700.
[7] C. Bormann, S. Mattern, H. Schrempf, H.P. Fiedler, H. Zahner, Isolation of *Streptomyces tendae* mutants with an altered nikkomycin spectrum, J. Antibiot. (Tokyo) 42 (1989) 913–918.
[8] C. Bruntner, C. Bormann, The *Streptomyces tendae* Tü901 L-lysine 2-aminotransferase catalyzes the initial reaction in nikkomycin D biosynthesis, Eur. J. Biochem. 254 (1998) 347–355.
[9] D. Venci, G. Zhao, M.S. Jorns, Molecular characterization of NikD, a new flavoenzyme important in the biosynthesis of nikkomycin antibiotics, Biochemistry 41 (2002) 15795–15802.
[10] G. Niu, G. Liu, Y. Tian, H. Tan, SanJ, an ATP-dependent picolinate–CoA ligase, catalyzes the conversion of picolinate to picolinate–CoA during nikkomycin biosynthesis in *Streptomyces ansochromogenes*, Metab. Eng. 8 (2006) 183–195.
[11] Y. Li, H. Ling, W. Li, H. Tan, Improvement of nikkomycin production by enhanced copy of *sanU* and *sanV* in *Streptomyces ansochromogenes* and characterization of a novel glutamate mutase encoded by *sanU* and *sanV*, Metab. Eng. 7 (2005) 165–173.
[12] C. Bruntner, B. Lauer, W. Schwarz, V. Mohrle, C. Bormann, Molecular characterization of co-transcribed genes from *Streptomyces tendae* Tü901 involved in the biosynthesis of the peptidyl moiety of the peptidyl nucleoside antibiotic nikkomycin, Mol. Gen. Genet. 262 (1999) 102–114.
[13] I. Hinterlang, Isolierung, Strukturaufklärung und Stoffwechsel eines Picolinsäuremetaboliten aus *Streptomyces tendae*. Ph.D. thesis, Universität Münster, 1992.
[14] G. Liu, Y. Tian, H. Yang, H. Tan, A pathway-specific transcriptional regulatory gene for nikkomycin biosynthesis in *Streptomyces ansochromogenes* that also influences colony development, Mol. Microbiol. 55 (2005) 1855–1866.
[15] T. Kieser, M.J. Bibb, M.J. Buttner, K.F. Chater, D.A. Hopwood, Practical Streptomyces Genetics, The John Innes Foundation, Norwich, UK, 2000.
[16] D.J. MacNeil, K.M. Gewain, C.L. Ruby, G. Dezeny, P.H. Gibbons, T. MacNeil, Analysis of *Streptomyces avermitilis* genes required for avermectin biosynthesis utilizing a novel integration vector, Gene 111 (1992) 61–68.
[17] M.S. Paget, L. Chamberlin, A. Atrih, S.J. Foster, M.J. Buttner, Evidence that the extracytoplasmic function sigma factor sigmaE is required for normal cell wall structure in *Streptomyces coelicolor* A3(2), J. Bacteriol. 181 (1999) 204–211.
[18] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, third ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
[19] S. Aemprapa, P.A. Williams, Implications of the *xylQ* gene of TOL plasmid pWW102 for the evolution of aromatic catabolic pathways, Microbiology 144 (1998) 1387–1396.
[20] M. Tsuda, H. Genka, Identification and characterization of Tn4656, a novel class II transposon carrying a set of toluene-degrading genes from TOL plasmid pWW53, J. Bacteriol. 183 (2001) 6215–6224.
[21] D. Miyazawa, G. Mukerjee-Dhar, M. Shimura, T. Hatta, K. Kimbara, Genes for Mn(II)-dependent NahC and Fe(II)-dependent NahH located in close proximity in the thermophilic naphthalene and PCB degrader, *Bacillus sp*. JF8: cloning and characterization, Microbiology 150 (2004) 993–1004.
[22] H. Arai, T. Ohishi, M.Y. Chang, T. Kudo, Arrangement and regulation of the genes for meta-pathway enzymes required for degradation of phenol in *Comamonas testosteroni* TA441, Microbiology 146 (Pt 7) (2000) 1707–1715.
[23] T. Muraki, M. Taki, Y. Hasegawa, H. Iwaki, P.C.K. Lau, Prokaryotic homologs of the eukaryotic 3-hydroxyanthranilate 3,4-dioxygenase and 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase in the 2-nitrobenzoate degradation pathway of *Pseudomonas fluorescens* strain KU-7, Appl. Environ. Microbiol. 69 (2003) 1564–1572.
[24] B.A. Manjasetty, J. Powlowski, A. Vrielink, Crystal structure of a bifunctional aldolase–dehydrogenase: sequestering a reactive and volatile intermediate, Proc. Natl. Acad. Sci. USA 100 (2003) 6992–6997.
[25] S.J. Lee, J.H. Ko, H.Y. Kang, Y. Lee, Coupled expression of MhpE aldolase and MhpF dehydrogenase in *Escherichia coli*, Biochem. Biophys. Res. Commun. 346 (2006) 1009–1015.
[26] A. Platt, V. Shingler, S.C. Taylor, P.A. Williams, The 4-hydroxy-2-oxovalerate aldolase and acetaldehyde dehydrogenase (acylating) encoded by the *nahM* and *nahO* genes of the naphthalene catabolic plasmid pWW60-22 provide further evidence of conservation of meta-cleavage pathway gene sequences, Microbiology 141 (Pt 9) (1995) 2223–2233.
[27] J. Powlowski, L. Sahlman, V. Shingler, Purification and properties of the physically associated meta-cleavage pathway enzymes 4-hydroxy-2-ketovalerate aldolase and aldehyde dehydrogenase (acylating) from *Pseudomonas sp*. strain CF600, J. Bacteriol. 175 (1993) 377–385.