Selectively improving nikkomycin Z production by blocking the imidazolone biosynthetic pathway of nikkomycin X and uracil feeding in Streptomyces ansochromogenes

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Abstract

Background: Nikkomycins are a group of peptidyl nucleoside antibiotics and act as potent inhibitors of chitin synthases in fungi and insects. Nikkomycin X and Z are the main components produced by Streptomyces ansochromogenes. Of them, nikkomycin Z is a promising antifungal agent with clinical significance. Since highly structural similarities between nikkomycin Z and X, separation of nikkomycin Z from the culture medium of S. ansochromogenes is difficult. Thus, generating a nikkomycin Z selectively producing strain is vital to scale up the nikkomycin Z yields for clinical trials.

Results: A nikkomycin Z producing strain (sanPDM) was constructed by blocking the imidazolone biosynthetic pathway of nikkomycin X via genetic manipulation and yielded 300 mg/L nikkomycin Z and abolished the nikkomycin X production. To further increase the yield of nikkomycin Z, the effects of different precursors on its production were investigated. Precursors of nucleoside moiety (uracil or uridine) had a stimulatory effect on nikkomycin Z production while precursors of peptidyl moiety (L-lysine and L-glutamate) had no effect. sanPDM produced the maximum yields of nikkomycin Z (800 mg/L) in the presence of uracil at the concentration of 2 g/L and it was approximately 2.6-fold higher than that of the parent strain.

Conclusion: A high nikkomycin Z selectively producing was obtained by genetic manipulation combined with precursors feeding. The strategy presented here might be applicable in other bacteria to selectively produce targeted antibiotics.

Background

Nikkomycins, a group of peptidyl nucleoside antibiotics produced by Streptomyces ansochromogenes [1] and Streptomyces tendae [2], are potent competitive inhibitors of chitin synthase. These antibiotics are structurally similar to UDP-N-acetylglucosamine which is the natural substrate of chitin synthase. So they can inhibit the growth of insects, acarids, yeasts, and filamentous fungi [3]. Nikkomycin X and Z, main components produced by both S. ansochromogenes and S. tendae, are the most active struc-
tures (Fig. 1). They are composed of hydroxypyridyl-homethreonine (nikkomycin D) and a 5-aminohexuronic acid N-glucosidically bound to uracil in nikkomycin Z or to 4-formyl-4-imidazolin-2-one (imidazolone) in nikkomycin X. The corresponding nucleoside moieties are designated as nikkomycin Cz and Cx. Nikkomycin I and J, produced as minor components by S. tendae but not by S. ansochromogenes, are structurally analogous to nikkomycin X and nikkomycin Z and contain glutamic acid peptidically bound to the 6'-carboxyl group of aminoheuronic acid [4]. In the past few years, particular attention has been drawn to nikkomycin Z for its significant activity against the highly chitinous, pathogenic, dimorphic fungi Coccidioides immitis and Blastomyces dermatitidis and phase I/II clinical research of nikkomycin Z as an orphan product for treatment of coccidiodomycosis is undergoing [5]. Meanwhile, nikkomycin Z has synergetic effect with azoles and echinocandins against Candida albicas and Aspergillus fumigatus [6-8].

Separation of nikkomycin Z from the culture medium is difficult due to the highly structural similarity among nikkomycins. This is much more complicated by its isomer nikkomycin X. Thus, the abolition of nikkomycin X, I and J production is crucial for scaling up nikkomycin Z yields for clinical trials. Studies such as strain improvement, optimization of the production medium and fermentation process, have significantly increased the yield of nikkomycins, but strains selectively producing nikkomycin Z remain unavailable [9]. Recently, considerable progresses have been made in understanding nikkomycin biosynthesis in S. ansochromogenes and S. tendae. Nikkomycin biosynthetic cluster has been identified in both strains and subsequent biochemical characterizations have elucidated the functions of some genes. Among them, sanO, sanQ, sanR and SanX were involved in biosynthesis of nikkomycin Cx and Cz (Fig. 2) [10-12]. Gene disruption of sanO or sanQ resulted in the blocking of nikkomycin X biosynthesis in S. ansochromogenes 7100, but had no effect on the production of nikkomycin Z. These studies raised the possibility that the blocking of nikkomycin X biosynthesis by genetic manipulation in S. ansochromogenes might generate a dedicated nikkomycin Z producing strain, since nikkomycin I and J were not produced by S. ansochromogenes.

In this paper, a strain which produced high-level of nikkomycins obtained by traditional strain improvement was chosen as the parent strain for genetic manipulation. A nikkomycin Z selectively producing strain was generated by blocking the imidazolone biosynthetic pathway of nikkomycin X. The subsequent uracil feeding further enhanced the yield of nikkomycin Z.

Results

Construction of the nikkomycin Z selectively producing strain

To obtain an ideal strain only producing nikkomycin Z, the disruption of sanP in S. ansochromogenes TH322 was performed (Fig. 3A). sanP is an homologue of nkp2 of S. tendae (sharing 95% identity), which is vital for the biosynthesis of imidazolone that is a unique part of nikkomycin X [13]. The resulting sanP disruption mutant (sanPDM) was passed through five generations in the absence of antibiotic pressure. The progeny still conferred kanamycin resistance, indicating the resulting sanPDM was genetically stable.

S. ansochromogenes sanPDM produced almost the same amounts of nikkomycin Z (300 mg/L) as TH322 (Fig. 3B), while no nikkomycin X was produced by sanPDM. Biomass of sanPDM was approximately equal to that of TH322 (Fig. 4A).

In S. ansochromogenes, sanP is located in one of the operons [1]. To exclude the potential polar effect of sanP disruption on the loss of nikkomycin X, the complementary plasmid plM229::sanP constructed with wild type sanP under the control of the ermE* promoter was introduced into the sanPDM. The introduction of plM229::sanP into sanPDM restored nikkomycin X production. HPLC analysis and bioassay showed that the amounts of nikkomycins produced by the complemented strain were almost the same as those produced by the parent strain (Fig. 3B).
disruption and complementation experiments suggested that sanP was a key determinant in nikkomycin X biosynthesis and disruption of sanP did not affect the production of nikkomycin Z.

Effect of precursor of nucleoside moiety on nikkomycin Z production
To further increase the yield of nikkomycin Z, the effects of different precursors on its production were investigated. Recent studies revealed that UMP was the direct precursor for the biosynthesis of the nucleoside moiety of nikkomycin Z [14] and UMP could be synthesized from uracil or uridine through salvage pathway. So, the effect of supplementation of varying concentration (1 g/L to 3 g/L) of uracil on nikkomycin production was measured in S. ansochromogenes TH322 and sanPDM. As shown in Fig. 4B, uracil had a stimulatory effect on nikkomycin Z production in both strains and uracil supplementation led to much more nikkomycin Z production in sanPDM than that in TH322 at all concentrations tested in the study.

Addition of uracil (1 g/L to 2 g/L) resulted in increased nikkomycin Z production from 450 mg/L to 800 mg/L in sanPDM or increased from 375 mg/L to 630 mg/L in TH322, whereas nikkomycin X production was decreased from 195 mg/L to 96 mg/L in TH322 (Fig. 4C). The change of biomass was observed in both strains. Addition of uracil (2 g/L) in the culture medium resulted in reduced the biomass by approximate 40% compared to that of the control (Fig. 4A). Addition of more uracil (3 g/L) resulted in significant inhibition of cell growth and reduced the final yield of nikkomycins in both strains. These results demonstrated that addition higher concentration of uracil might inhibit cell growth.

To determine uracil consumption, uracil residue in the culture medium after fermentation was measured. As shown in Table 1, uracil could be hardly detected when uracil was fed at the concentration of 1.5 g/L, while 0.484 g/L uracil left when 2 g/L uracil was fed. These results indicated that only 1.5 g/L uracil can be utilized during the fermentation.
mentation. In the subsequent experiments, a saturated concentration of uracil (2 g/L) was used to achieve the maximum production of nikkomycin Z.

Similar studies were carried out to determine the effect of uridine supplementation on nikkomycin Z production. Addition of uridine (0.5 g/L to 2 g/L) resulted in enhancing nikkomycin Z production from 320 mg/L to 400 mg/L without detectable effect on the biomass (data not shown). Thus, uridine exerted a less stimulatory effect than uracil on nikkomycin Z production.

![Figure 3](image-url)  
**Figure 3**  
Insertional inactivation of sanP and analysis of nikkomycin production. (A) Diagram of sanP gene disruption in *S. ansochromogenes* TH322. A kanamycin antibiotic resistance cassette was inserted into the BamHI site of sanP. (B) Bioassay and HPLC analysis of nikkomycin production. X, nikkomycin X; Z, nikkomycin Z; 1, culture filtrates from TH322; 2, culture filtrates from sanPDM; 3, culture filtrates from the complementary strain; 4, culture filtrates from sanPDM feeding with 2 g/L uracil. The strains were inoculated in liquid SP medium for 144 h.

![Figure 4](image-url)  
**Figure 4**  
Effect of uracil on biomass and nikkomycin production. (A) Effect of uracil on the biomass of *S. ansochromogenes* TH322 and sanPDM; (B) Effect of uracil on the nikkomycin Z production in *S. ansochromogenes* TH322 and sanPDM; (C) Effect of uracil on the nikkomycin X production in *S. ansochromogenes* TH322.
Effect of precursor of peptidyl moiety on nikkomycin Z production

Precursor feeding and biochemical studies have shown that L-glutamate and L-lysine are precursors for biosynthesis of peptidyl moiety of nikkomycin Z [15,16]. L-lysine and L-glutamate (0.5, 1 and 2 g/L) were added separately to the culture medium fed with 2 g/L uracil in order to study their influences on the nikkomycin Z production in S. ansochromogenes sanPDM. It was observed that L-lysine (0.5 g/L to 1 g/L) had no remarkable effect on the nikkomycin Z production and the biomass (Table 2). Further increasing the concentration of lysine resulted in decreased the yield of nikkomycin Z and inhibited the cell growth. Similar results were obtained when L-glutamate was added into the culture medium. As both L-lysine and L-glutamate are needed to synthesize the peptidyl moiety, the effect of supplementing L-lysine and L-glutamate in culture medium on the yield of nikkomycin Z was studied (Table 2). It was observed that there was no increase in nikkomycin Z production with L-lysine and L-glutamate feeding compared to that without supplementation. These results together demonstrated that L-lysine and L-glutamate might not be the limiting-factor of nikkomycin Z production in the complex SP medium. So, the optimum condition to obtain maximum productivity of nikkomycin Z (800 mg/L) was culturing the sanPDM in the presence of uracil at the concentration of 2 g/L.

Discussion

Nikkomycin Z is a potential antifungal agent with clinical significance. However, the presence of its isomer, nikkomycin X during fermentation interferes with the purification of nikkomycin Z and increases the production cost. Thus, we chose a nikkomycin high producing strain (S. ansochromogenes TH322) to perform genetic manipulation and obtained a nikkomycin Z selectively producing strain (sanPDM). sanP encodes a type II thioesterase which hydrolytically released SanO from β-OH-His-SanO to form β-OH-His (Fig. 2). Disruption of it resulted in blocking the synthesis of β-OH-His and failed production of nikkomycin Cx and nikkomycin X, whereas, disruption of sanP did not affect nikkomycin Z production because uracil, the base in nikkomycin Z, was biosynthesized via de novo or salvage pathway. These results were mainly in accordance with those studies in S. tendae [13]. Disruption of nikP2 almost abolished the nikkomycin X production, while disruption of sanP in S. ansochromogenes resulted in eliminating nikkomycin X production. Loss of the nikkomycin X production in the fermentation would significantly improve the recovery of nikkomycin Z during large scale manufacturing process.

Feeding precursors have been proved to be a successful strategy to increase the yield of targeted antibiotics, such as feeding ornithine to enhance clavulanic acid production in S. clavuligerus [17]. We found that addition of uracil (2 g/L) increased production of nikkomycin Z by 2-fold while suppressed nikkomycin X production by 85% in TH322. A possible explanation to these results was that UMP derived form uracil competed with imidazolone for incorporation into core of nikkomycin. A similar study on salinosporamide B in Salinispora tropica was verified, finding that addition of butyric acid-the precursor of salinosporamide B to the culture medium resulted in increased the production of salinosporamide B by approximately 3.2-fold while inhibited the production of salinosporamide A by 26% [18].
Uracil supplementation led to considerable increase of nikkomycin Z production, while its high concentration (more than 2 g/L) significantly inhibited cell growth and reduced the yield of nikkomycin Z. Our results were in accordance with the reports of Krishna [19]. Uracil (2 g/L) could improve the rifamycin SV production by 505 mg/L in Amycolatopsis mediterranei MV35R, while higher concentration inhibited both the cell growth and rifamycin SV production. The reason why higher concentration of uracil sharply inhibited the cell growth was still unclear. Uridine had a slight effect on nikkomycin Z production. Uracil salvage pathway had been identified in S. gries and S. coelicolor [20] and genes involving in uracil transport (uracil permase) and transformation to UMP (uracil phosphoribosyltransferase) were highly conserved in Streptomyces. However, gene encoding uridine kinase that converted uridine into UMP had not been found in Streptomyces so far. So, we speculated that the stimulatory effect of uridine on nikkomycin Z production may be due to the partial degradation of uridine to uracil and uracil acted as precursor to participate in nikkomycin Z biosynthesis.

**Conclusion**

Combining genetic manipulation with uracil feeding enabled us to selectively enhance the yield of nikkomycin Z. As nikkomycin Z could be recovered without additional separation from other related components in sanPDM, it would simplify the downstream purification process and lower the production cost.

**Methods**

**Strains, plasmids and culture conditions**

Streptomyces ansochromogenes TH322, a high-level producer of nikkomycins (300 mg/L nikkomycin Z and 600 mg/L nikkomycin X), was collected in our laboratory. S. ansochromogenes sanPDM, the nikkomycin Z selectively producing strain created in this study was deposited with the China General Microbiological Culture Collection Center (CGMCC) and assigned the accession number was CGMCC-3086. Candida albicans CGMCC No 2.2086 was used as indicator strain for nikkomycin bioassays. Escherichia coli JM109 was used as host for cloning and subcloning. E. coli ET12567 containing pUZ8002 was used for conjugal transfer according to the established protocol [21]. Streptomyces-E. coli shuttle plasmid pKC1139 for gene disruption was provided by Prof. Keith Chater (John Innes Center, Norwich, UK)

S. ansochromogenes TH322 was grown on mannitol/soya (MS) medium at 28°C [21] for 6 days to form spores. For genomic DNA isolation, the S. ansochromogenes TH322 and sanP disruption mutant (sanPDM) were grown in YEME medium for 2 days. SP medium (3% mannitol, 1% soluble starch, 0.5% soy peptone and 0.8% yeast extract, pH 6.0) was used for nikkomycin production.

When necessary, antibiotics were used at the following concentrations: apramycin, 10 μg ml⁻¹ in YEME or MS for S. ansochromogenes TH322, 100 μg ml⁻¹ in LB for E. coli; kanamycin, 10 μg ml⁻¹ in YEME or MS for S. ansochromogenes TH322, 100 μg ml⁻¹ in LB for E. coli.

**Primers and PCR conditions**

The oligonucleotide primers used to amplify the sanP were P1 (5'-GTGGCAGCGCCAGGGCG-3') and P2 (5'-TCAACTCCTCATCGGCTC-3'). P3 (5'-GCGGCCAGCTACTTCCGGGAC-3') and P4 (5'-GCAGAAGCGCCAGGCATGT-3') were used to check the sanP disruption mutant. The PCR was performed using KOD (Toyobo, Japan) an initial denaturation at 95°C for 4 min followed by 30 cycles of amplification (95°C for 1 min, 62°C for 30 sec, and 68°C for 1 min) and additional 10 min at 68°C.

**Construction of sanP disruption mutants**

Disruption of sanP was performed by gene replacement via homologous recombination. For this purpose, a 2.5 kb PstI-SacI DNA fragment containing the complete sanP sequence was inserted into the PstI-SacI site of pBluescript II KS (-) to generate pBS::sanP. The kanamycin-resistance gene (neo) was isolated from pUC119::neo after digestion with BamHI and KpnI, blunted and inserted into the blunted BamHI site of sanP in pBS::sanP to generate pBS::sanP::neo. A 3.5 kb PstI-SacI fragment from pBS::sanP::neo was inserted into the PstI-SacI site of pJJ2925 to give pJJ2925::sanP::neo. The resulting plasmid was then digested with BglII and the resulting 3.5 kb fragment was inserted into the BamHI site of pKC1139 to generate pKC1139::sanP::neo.

The resulting plasmid pKC1139::sanP::neo was passed through E. coli ET12567/pUZ8002 and then introduced into S. ansochromogenes TH322 by conjugal transfer according to the established techniques. The resulting transformants were inoculated on MS plates to form spores. Gray spores were harvested and spread on MM plates containing kanamycin as the resistance selection. After incubating at 42°C for 3 days, the colonies that conferred kanamycin resistance (KanR) and apramycin sensitivity (AprS) were selected and further confirmed as sanP disruption mutants by PCR using the sanPDM genomic DNA as template.

**Complementation of the sanP mutant**

For complementation analysis, complete sanP DNA fragment was amplified from S. ansochromogenes TH322 genomic DNA by PCR using P1 and P2 primers. The amplified fragment was subsequently inserted into the EcoRV site of pLM229 under the control of promoter of ermE* [22]. The resulting recombinant plasmid
pLM229::sanP was then integrated into the chromosomal attB site of sanP disruption mutant by conjugal transfer.

**Fermentation of S. ansocromogenes**

Spores of *S. anchro- gromogenes TH322 or sanPDM were inoculated in YEME. The cultures were grown at 28°C on a rotary shaker (220 rpm) for 48 h and used as seed cultures. 1 ml (0.5% V/V) of seed culture was inoculated into flasks containing 50 ml of SP medium, and then fermented at 28°C on a rotary shaker (200 r.p.m) for 6 days.

**Quantification of uracil in the culture filtrates**

Uracil was analyzed according to the method of Gao [23] by HPLC using a Zorbax SB-C18 reverse-phase column at 254 nm with a 0.5 ml min⁻¹ flow rate at 25°C. The elution profile was a linear gradient of 0%-20% solution B (A = 5 mM ammonium acetate; B = methanol) over 15 min. An aliquot of 20 μl culture filtrates was injected for HPLC analysis.

**Bioassay and HPLC analysis of nikkomycins**

The culture filtrates were harvested by centrifugation and the supernatant was filtered through a Minipore membrane (pore diameter 0.2 μm). Nikkomycin Z (Sigma, cat. No. N8028) was used as standard. HPLC analysis and bioassay of nikkomycins against *Candida albicans* were performed as described previously [24].

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

GL carried out the experiments, wrote the draft manuscript and analyzed the primary data. JL, LL, HY and YT assisted with experimental design, data analysis. HT supervised the whole work and revised the manuscript. All authors read and approved the final manuscript.

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**References**

1. Liu G, Tian Y, Yang H, Tan H: A pathway-specific transcriptional regulatory gene for nikkomycin biosynthesis in Streptomyces ansocromogenes that also influences colony development. *Mal Microbiol* 2005, 55(6):1855-1866.

2. Mohrle V, Roos U, Bormann C: Identification of cellular proteins involved in nikkomycin production in Streptomyces tendae Tu901. *Mal Microbiol* 1995, 15(5):561-571.

3. Hector RF: Compounds active against cell walls of medically important fungi. *Clin Microbiol Rev* 1993, 6(1):1-21.

4. Decker H, Bormann C, Fiedler HP, Zahner H, Heitsch H, König WA: Metabolic products of microorganisms. 252: Isolation of new nikkomycins from Streptomyces tendae. J Antibiot (Tokyo) 1989, 42(2):230-235.

5. Nix DE, Sweezy RR, Hector R, Galgani JN: Pharmacokinetics of Nikkomycin Z after Single Rising Oral Doses. *Antimicrob Agents Chemother*. Epub 2009

6. Ganesan LT, Manavathu EK, Cutright JL, Alangaden GJ, Chandrasekar PV: In-vitro activity of nikkomycin Z alone and in combination with polyenes, triazoles or echinocandins against *Aspergillus fumigatus*. *Clin Microbiol Infect* 2004, 10(11):961-966.

7. Luque JC, Clemens KV, Stevens DA: Efficacy of micafungin alone or in combination against systemic murine aspergillosis. *Antimicrob Agents Chemother* 2003, 47(4):1452-1455.

8. Sandovsky-Losica H, Swartzman R, Lahar Y, Segal E: Antifungal activity against Candida albicans of nikkomycin Z in combination with caspofungin, voriconazole or amphotericin B. *J Antimicrob Chemother* 2006, 62(3):365-367.

9. Fiedler H-P, Schutz U, Decke H: An overview of nikkomycins: history, biochemistry and applications. In *Cutaneous antifungal agents*: selected compounds in clinical practice and development. Edited by: Rippon JW, Fromting LA. New York: Marcel Dekker, Inc; 1993:325-352.

10. Li W: Structure and Function of Gene Involved in Nikkomycin Biosynthesis and Differentiation in *Streptomyces ansocromogenes*. In PhD Dissertation Beijing: Institute of microbiology, CAS; 2003.

11. Wang G, Nie L, Tan H: Cloning and characterization of sanO, a gene involved in nikkomycin biosynthesis in *Streptomyces ansocromogenes*. Let Appl Microbiol 2003, 37(6):452-457.

12. Zeng H, Tan H, Li J: Cloning and function of sanQ: a gene involved in nikkomycin biosynthesis of *Streptomyces ansocromogenes*. *Curr Microbiol* 2002, 45(3):175-179.

13. Lauer B, Russwurm R, Schwarz W, Kalmanczelyi A, Brunnter C, Rosemeier A, Bormann C: Molecular characterization of co-transcribed genes from Streptomyces tendae Tu901 involved in the biosynthesis of the peptide moiety and assembly of the peptidyl nucleoside antibiotic nikkomycin. *Mal Gen Genet* 2001, 26(5):662-673.

14. Ginj C, Ruegger H, Amrhein N, Macheroux P: 3'-Enolpyruvyl-UMP, a novel and unexpected metabolite in nikkomycin biosynthesis. *ChemBiochem* 2005, 6(11):1974-1976.

15. Li Y, Ling H, Li W, Tan H: Improvement of nikkomycin production by enhanced copy of sanU and sanV in *Streptomyces ansocromogenes* and characterization of a novel glutamate mutase encoded by sanU and sanV. *Metab Eng* 2005, 7(3):165-173.

16. Brunnter C, Bormann C: The Streptomyces tendae Tu901 L-lysine aminotransferase catalyzes the initial reaction in nikkomycin D biosynthesis. *Eur J Biochem* 1998, 254(2):347-355.

17. Romero J, Liras P, Martin JF: Utilization of orithnine and arginine as specific precursors of clavulanic acid. *Appl Environ Microbiol* 1986, 52(4):892-897.

18. Tsueng G, McArthur KA, Poets BC, Lam KS: Unique butyric acid incorporation patterns for salinosporamide A and B reveal distinct biosynthetic origins. *Appl Microbial Biotechnol* 2007, 75(5):999-1005.

19. Murai Krishna PS, Venkateswarlu G, Venkateswar Rao L: Effect of uracil on rifamycin SV production by *Amycolatopsis mediterranei* MV35R. *Let Appl Microbiol* 2000, 31(1):73-76.

20. Hughes LE, Beck DA, O’Donovan GA: Pathways of pyrimidine salvage in *Streptomyces*. *Curr Microbiol* 2005, 50(1):8-10.

21. Kieser T, Bibb MJ, Butterler MJ, Chater KF, Hopwood DA: Practical *Streptomyces* Genetics. John Innes Foundation, Norwich, England; 2000.

22. Zheng JT, Wang SL, Yang KQ: Engineering a regulatory region of jadomycin gene cluster to improve jadomycin B production in *Streptomyces venezuelae*. *Appl Microbial Biotechnol* 2007, 76(4):883-888.

23. Gao JL, Leung KS, Wang YJ, Lai CM, Li SP, Hu LF, Lu GH, Jiang ZH, Yu ZL: Qualitative and quantitative analyses of nucleosides from *Ganoderma spp.* by HPLC-DAD-MS. *J Pharm Biomed Anal* 2007, 44(3):807-819.

24. Niu G, Liu G, Tian Y, Tan H, Sanj, an ATP-dependent picolinate-CoA ligase, catalyzes the conversion of picolinate to picolinate-CoA during nikkomycin biosynthesis in *Streptomyces ansocromogenes*. *Metab Eng* 2006, 8(3):183-195.