Identification of novel tylosin analogues generated by a \textit{wblA} disruption mutant of \textit{Streptomyces ansochromogenes}

Cheng Lu$^{1,2}$, Guojian Liao$^{3}$, Jihui Zhang$^{1,*}$ and Huarong Tan$^{1,*}$

Abstract

\textbf{Background:} \textit{Streptomyces}, as the main source of antibiotics, has been intensively exploited for discovering new drug candidates to combat the evolving pathogens. Disruption of \textit{wblA}, an actinobacteria-specific gene controlling major developmental transition, can cause the alteration of phenotype and morphology in many species of \textit{Streptomyces}. One \textit{wblA} homologue was found in \textit{Streptomyces ansochromogenes} 7100 by using the Basic Local Alignment Search Tool. It is interesting to identify whether novel secondary metabolites could be produced by the \textit{wblA} disruption mutant as evidenced in other \textit{Streptomyces}.

\textbf{Results:} The \textit{wblA} disruption mutant of \textit{S. ansochromogenes} 7100 (Δ\textit{wblA}) was constructed by homologous recombination. Δ\textit{wblA} failed to produce spores and nikkomycin, the major product of \textit{S. ansochromogenes} 7100 (wild-type strain) during fermentation. Antibacterial activity against \textit{Staphylococcus aureus} and \textit{Bacillus cereus} was observed with fermentation broth of Δ\textit{wblA} but not with that of the wild-type strain. To identify the antibacterial compounds, the two compounds (compound 1 and compound 2) produced by Δ\textit{wblA} were characterized as 16-membered macrolides by mass spectrometry and nuclear magnetic resonance spectroscopy. The chemical structure of these compounds shows similarity with tylosin, and the bioassays indicated that the two compounds inhibited the growth of a number of gram-positive bacteria. It is intriguing that they displayed much higher activity than tylosin against \textit{Streptococcus pneumoniae}.

\textbf{Conclusions:} Two novel tylosin analogues (compound 1 and 2) were generated by Δ\textit{wblA}. Bioassays showed that compound 1 and 2 displayed much higher activity than tylosin against \textit{Streptococcus pneumoniae}, implying that these two compounds might be used to widen the application of tylosin.

\textbf{Keywords:} \textit{wblA}, Nikkomycin, Tylosin analogues, \textit{Streptomyces ansochromogenes}, Bioassay

Background

The crisis of antibiotic resistance has become an impending global problem, so novel antibiotics are required to combat the evolving pathogens and new emerging diseases. More than half of medically important antimicrobial and antitumor antibiotics are produced by \textit{Streptomyces}. Genome engineering and gene manipulation on secondary metabolic gene clusters have been widely applied for exploring novel bioactive agents. For example, using heterologous expression, a 157 kb daptomycin biosynthetic gene cluster from \textit{Streptomyces roseosporus} NRRL 15998 was successfully cloned and heterologously expressed in \textit{Streptomyces coelicolor} [1]. Two hybrid antibiotics were generated by genetic manipulation of the nikkomycin and polyoxin biosynthetic gene clusters [2]. Supplementation of the mutasynthesis strain with nicotinic acid led to the production of two novel nikkomycin analogues [3]. However, sequencing of several \textit{Streptomyces} genomes revealed that a large number of antibiotic biosynthetic gene clusters are present, which have the potential to produce many more natural products than had previously been recognized [4–7].

*Correspondence: zhang.jihui@im.ac.cn; tanhr@im.ac.cn

1 State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

Full list of author information is available at the end of the article

© 2015 Lu et al. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Therefore, it has become necessary to devise methods and strategies to identify valuable natural products. One of the features of antibiotic synthesis in Streptomyces is that the production of antibiotics is generally associated with the development and differentiation of Streptomyces. Genetic manipulations of pleiotropic regulators responsible for both differentiation and antibiotic production may effectively influence the expression of certain genes involved in metabolic pathways, thus it would be an efficient strategy for searching novel metabolites. By this approach, comprehensive elucidations on biosynthetic pathways or regulatory mechanisms of the metabolite biosynthesis could be circumvented.

whi genes are involved in the life cycle of Streptomyces as well as in the production of various antibiotics [8]. Disruption of these genes resulted in white phenotype of aerial hyphae in Streptomyces, so they were named as whi genes. whiB gene was originally discovered in Streptomyces coelicolor, and whiB-like (wbl) genes are widespread in Streptomyces [9, 10]. There are at least 11 homologues of whiB genes on the chromosome of S. coelicolor. Mutation or absence of wblA caused multiple effects on Streptomyces, such as the failure of sporulation, enhancement of actinorhodin, undecylprodigiosin, doxorubicin, tautomycin, and moenomycin production [9, 11–13]. Therefore, WblA is recognized as a global regulator. It plays as a repressor of antibiotic production in S. coelicolor, but acts as a pivotal activator for natamycin biosynthesis in Streptomyces chattanoogensis L10 [14]. Streptomyces ansochromogenes 7100, a natural peptidyl nucleoside antibiotic nikkomycin producer, has been studied for decades [15]. Like other well-studied Streptomyces, it has a typical life cycle of differentiation and development with aerial mycelia and spore formation accompanied by secondary metabolites biosynthesis. In search of the sequenced genome of S. ansochromogenes, a whiB-like gene situated on the chromosome was found and its encoding protein shares 96 % identity with WblA in S. coelicolor, likewise it was named as wblA (gene accession number KT583835).

In this study, we focused on the secondary metabolites produced by the wblA disruption mutant of S. ansochromogenes 7100 (ΔwblA). It is intriguing that ΔwblA failed to produce nikkomycin but led to the discovery of novel active metabolites simultaneously. These compounds were subsequently isolated, purified and analyzed for their structures and bioactivities against a number of bacteria.

### Results

#### Construction of wblA disruption mutant and its complementation

In order to identify whether the metabolite profile could be affected by wblA disruption in S. ansochromogenes 7100, ΔwblA was constructed via homologous recombination. As expected, ΔwblA failed to form grey spores and spore chains on minimal medium (MM) agar in comparison with wild-type (WT) strain (Fig. 1a–c). On the other hand, nikkomycin, the only secondary metabolite identified so far from this strain, was examined. Cultures from the same time-course experiments were subjected to bioassays against Alternaria longipes and Candida albicans for nikkomycin activity test (Fig. 2a, b). In contrast to WT strain, no inhibition zone was observed against above two indicator strains with the fermentation filtrate of ΔwblA. High-performance liquid chromatography (HPLC) analysis demonstrated that the production of nikkomycin was completely abolished in ΔwblA (Fig. 2c). To further verify the effect of wblA disruption on nikkomycin production, the transcription profile of genes involved in nikkomycin biosynthesis was analyzed by quantitative Real Time Polymerase Chain Reaction (qRT-PCR). The biosynthetic gene cluster of nikkomycin includes one pathway-specific regulatory gene (sanG) and 21 structural genes consisting of three transcriptional units (sanO-V, sanN-I and sanF-X) [16]. The first gene of each transcriptional unit was chosen to examine the transcription of corresponding genes. The results showed that transcriptions of sanG and other three genes (sanN, sanO and sanF) situated in each transcriptional unit were all not detected in ΔwblA, whereas the transcription of hrdB as internal control, encoding the principal sigma-like factor, was not affected by the disruption of wblA (Fig. 2d). Complementary experiment was performed by integrating a copy of wblA and pSET152 vector into the chromosome of ΔwblA, respectively. As expected, nikkomycin production in ΔwblA was restored as that in WT strain (Fig. 2a–c). These results demonstrated that wblA is essential for nikkomycin biosynthesis in S. ansochromogenes 7100. Disruption of this gene affected not only the spore formation but also the nikkomycin biosynthesis, implying that wblA possesses multiple functions.

#### Analyses of the secondary metabolites of ΔwblA

Based on the fact that nikkomycin production was abolished in ΔwblA, it is noteworthy to identify whether new products could be produced by ΔwblA. The culture filtrates from the different time-course experiments were subjected to bioassays against representative gram-positive bacteria and gram-negative bacteria (Additional file 1: Table S1). The culture filtrate collected from ΔwblA after incubation for 96 h showed clear inhibition zones against both Staphylococcus aureus and Bacillus cereus, whereas no inhibition zone was found in the culture filtrate from WT (Fig. 3a, b). Chloroform extracts from these cultures were further analyzed by HPLC (Fig. 3c), and distinct peaks appeared at 17 min (compound I) and
18 min (compound 2) in the extract of ΔwblA (Fig. 4a). Both compounds gave rise to distinctive absorption at wavelength 286 nm on the ultra-violet (UV) spectra (Fig. 4b), indicating that they might be new products generated by ΔwblA since these two compounds were not found in WT under the same conditions.

**Isolation and structural analyses of compound 1 and 2**

To determine the chemical structures of compound 1 and 2, 18 liters of fermentation broth of ΔwblA in SP medium was harvested and extracted with chloroform. The organic phase was concentrated and applied onto Sephadex LH-20 column for further purification. 2.3 mg of compound 1 and 5.2 mg of compound 2 were obtained after final separation by semi-preparative HPLC. The chemical structures of these two compounds were determined by Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) spectroscopy.

High resolution positive-ion electron spray ionization mass spectrometry (HR-ESI–MS) of compound 1 gave a molecular ion peak at m/z 577.33459 ([M+Na–H₂O]⁺) and the molecular formula was found to be C_{29}H_{48}O_{11}. An initial survey of ¹H NMR and ¹³C NMR spectra (Fig. 5a, b) indicated the existence of two conjugated double bonds, a mycinose moiety and two carbonyl carbons (δ_C 174.7 and 204 ppm). Cross peaks between δ_H (4.98, 1.92 and 2.5 ppm) and δ_C 174.7 ppm on HMBC indicated that compound 1 could contain a macrolide backbone. Along with other correlations (Additional file 1: Figure S1) between protons and carbons on HMBC, HSQC and ¹H–¹H COSY, compound 1 seemed to be an analogue of tylosin, and most signals on the lactone and mycinose moiety could be assigned based on the NMR data of tylosin, except those at positions C5 and C6 [17]. Proton and carbon resonances for the two sugar moieties at C5 and acetaldehyde group at C6 in tylosin were absent, but two additional hydroxyl groups were present as indicated by the two sets of signals (δ_H 4.1, δ_C 71.6 ppm; δ_H 4.3, δ_C 67.9 ppm), which were determined by the following analysis. A cross peak between H4 (δ_H 1.48 ppm) and δ_H 4.1 ppm on ¹H–¹H COSY indicated that δ_H 4.1 ppm and δ_C 71.6 ppm could be assigned to C5; while the correlation between δ_H 4.3 ppm and δ_C 71.6 ppm on HMBC suggested that δ_H 4.3 ppm and δ_C 67.9 ppm could be
assigned to C6. Combined with other NMR data, compound 1 was determined as 6-hydroxy-21-O-mycinosyltylactone (Fig. 5c), a novel tylosin analogue.

For compound 2, HR-ESI–MS gave a molecular ion peak at $m/z$ 602.38965 ([M+NH$_4^+$]+) and the molecular formula was found to be C$_{31}$H$_{52}$O$_{10}$. Comparison of the $^1$H NMR and $^{13}$C NMR data (Fig. 5d, e) with those of compound 1 indicated a highly structural similarity between the two compounds, and the only difference is at C6. $\delta_H$ 4.3 ppm and $\delta_C$ 67.9 ppm at C6 were absent and the chemical shift at C6 was high-field shifted to $\delta_C$ 38 ppm in compound 2. Meanwhile, two sets of extra signals ($\delta_C$ 22.7 ppm and $\delta_H$ 1.62 ppm; $\delta_C$ 9.4 ppm and $\delta_H$ 0.92 ppm) showed the existence of an ethyl group, while the correlation between $\delta_H$ 0.92 ppm and C6 ($\delta_C$ 38 ppm) confirmed that the ethyl group is attached to C6. Further analysis of the HMBC, HSQC and COSY data (Additional file 1: Figure S2), compound 2 was determined as another tylosin analogue and designated as 23-O-mycinosyltylactone (Fig. 5f).

The NMR spectroscopic data of compound 1 and compound 2 are summarized in Table 1. The structural differences among compound 1, 2 and tylosin are illustrated (Fig. 5g).
Bioassays of compound 1 and 2

Structural elucidation showed compound 1 and compound 2 are 16-membered glycosylated macrolides. The functional groups responsible for the antibacterial activity of 16-membered macrolides are generally thought to be the aldehyde and the 9-keto group on

**Fig. 3** Bioassays and HPLC analysis of the fermentation broth from *S. anschromogenes* 7100 and ΔwblA. Bioassays of the fermentation broth against *Staphylococcus aureus* (a) and *Bacillus cereus* (b), and the HPLC analysis (c). (I): ΔwblA, (II): *S. anschromogenes* 7100, (III): complemented strain by integrating a copy of wblA into the chromosome of ΔwblA, (IV): the control strain by integrating pSET152 vector into the chromosome of ΔwblA. Arrows indicate the new appeared peaks on HPLC produced by ΔwblA.
the lactone, dimethylamino or methoxyl group on the sugar moieties and ethyl group at position C15 (Fig. 5g) [18]. In preliminary assays performed by disk diffusion tests, the fermentation filtrate of ΔwblA showed inhibitory activity against gram-positive pathogenic bacteria (Additional file 1: Table S1). MIC (minimum inhibitory concentration) values were then determined with purified compound 1 and compound 2 against a variety of gram-positive bacteria using tylosin as a control, a 16-membered macrolide antibiotic usually used in the treatment for various infections of animals [19, 20]. Compound 1 showed identical antimicrobial activity as compound 2, but their activity was less than that of tylosin against most of the tested strains (Table 2). All three compounds could not inhibit the growth of Staphylococcus epidermidis at 100 μg/ml. However, it is intriguing that compound 1 and compound 2 significantly inhibited the growth of S. pneumoniae and their MICs were more than ten folds lower than that of tylosin (Table 2). The results indicated that compound 1 and compound 2 are probably promising new derivatives of tylosin for further structural optimization.

**Discussion**

It is imperative to find novel families of antibiotics for tackling evolving pathogens. Streptomyces serves as the main source of antibiotics, despite most secondary metabolic pathways are silent or poorly expressed. Based on metabolic pathways and regulatory mechanisms of antibiotic biosynthesis, specific manipulation on key gene is feasible to redirect metabolic flux to the target metabolites, such as heterologous expression of the whole cluster, repressor deletion or activator enhancement, and so on. Those approaches enabled the discovery of numerous novel antibiotics [6]. However, a large proportion of secondary metabolic pathways in Streptomyces have not been unveiled. Therefore, it has become necessary to devise methods and strategies to identify these valuable secondary metabolites.

WblA of S. anschomrogenes 7100 shares 96 % sequence identity with that of S. coelicolor, and is a new member of pleiotropic regulators. Disruption of wblA influenced the morphological differentiation and the production of antibiotics in many Streptomyces spp. [13, 14]. As expected, the disruption of wblA in S. anschomrogenes...
7100 influenced spore formation and also abolished nikkomycin production, but led to the biosynthesis of two novel tylosin analogues. WblA can serve as a down-regulator or activator depending on the species of the strain probably via the iron-sulfur cluster in the molecule for sensing environmental signals, such as O₂ or nitric oxide [21, 22]. In S. ansochromogenes 7100, WblA exerted dual function in antibiotic biosynthesis, demonstrating that the regulators of this family play important roles. Other pleiotropic regulators widely exist in many species of Streptomyces, such as AdpA and BldA controlling more than one pathway [23, 24]. It is applicable to obtain new compounds from the cell secondary metabolite reservoir by disrupting a single pleiotropic gene without knowing details about the mechanism or the pathway of the metabolite biosynthesis. So far, exact regulatory mechanism of WblA and its orthologues regulating antibiotics biosynthesis are still unknown.

Fig. 5 Structural determinations of compound 1 and compound 2. a ¹H NMR of compound 1. b ¹³C NMR of compound 1. c The structure of compound 1. d ¹H NMR of compound 2. e ¹³C NMR of compound 2. f The structure of compound 2. g The structure of tylosin. Active groups contributing to tylosin activity are indicated by dashed line. The structural differences among tylosin, compound 1 and compound 2 are shown in red.
Table 1 Summary of 1H and 13C NMR data for compound 1 and compound 2 in CDCl3

| Position | Compound 1 | Compound 2 |
|----------|------------|------------|
|          | δ (1H, mult., J) | δ (1H, mult., J) | δ (13C) | δ (13C) |
| 1        | 1.92 (1H, d, 16) | 3.77 (1H, d, 10.0) | 174.7 | 174.7 |
| 2        | 2.5 (H, dd, 17, 10.7) | 1.48 (1H, *) | 39.2 | 39.2 |
| 3        | 3.72 (1H, d, 10.0) | 3.72 (1H, d, 10.0) | 67.1 | 66.8 |
| 4        | 1.48 (1H, *) | 1.48 (1H, *) | 39.9 | 39.9 |
| 5        | 4.1 (1H, d, 13.0) | 4.1 (1H, d, 9.0) | 71.6 | 72.6 |
| 6        | 4.3 (H, *) | 67.9 | 1.3 (H, *) | 38 |
| 7        | 2.0 (1H, m) | 2.0 (1H, m) | 29.2 | 32.7 |
| 8        | 1.56 (H, *) | 1.57 (H, *) | 39.2 | 45.1 |
| 9        | 2.8 (1H, br) | 2.8 (1H, br) | 45.1 | 45.1 |
| 10       | 6.34 (1H, d, 15.0) | 6.34 (1H, d, 15.0) | 118.3 | 118.6 |
| 11       | 7.32 (1H, d, 15.0) | 7.32 (1H, d, 15.0) | 148.1 | 147.8 |
| 12       | 135.1 | 135.1 | 135.5 |
| 13       | 5.92 (1H, d, 10.0) | 5.92 (1H, d, 10.0) | 141.9 | 141.7 |
| 14       | 2.98 (1H, m) | 2.98 (1H, m) | 45.1 | 45.1 |
| 15       | 4.98 (1H, ddd, 10.0, 10.2, 20) | 4.98 (1H, ddd, 10.0, 10.2, 20) | 75.3 | 75.2 |
| 16       | 1.88 (1H, m) | 1.88 (1H, m) | 25.4 | 25.5 |
| 17       | 1.63 (H, *) | 1.63 (H, *) | 1.63 (H, *) |
| 18       | 0.94 (H, s) | 0.94 (H, s) | 9.5 | 9.5 |
| 19       | 1.0 (3H, d, 6.0) | 1.0 (3H, d, 6.0) | 9.5 | 9.6 |
| 20       | 1.22 (3H, d, 7.0) | 1.62 (H, *) | 17.6 | 22.7 |
| 21       | 1.81 (3H, s) | 1.81 (3H, s) | 13.2 | 9.4 |
| 22       | 4.01 (1H, dd, 9.0, 4.0) | 4.01 (1H, dd, 9.0, 4.0) | 69.1 | 17.6 |
| 23       | 3.55 (H, s) | 3.55 (H, s) | 69.1 | 17.6 |

* Overlapping with other signals

Table 2 Antimicrobial activities of compound 1, 2 and tylosin

| Bacteria                        | MIC (μg/ml) |
|---------------------------------|-------------|
|                                 | Compound 1  | Compound 2  | Tylosin |
| Staphylococcus pneumoniae       | 7.06        | 7.31        | >100    |
| Streptococcus pyogenes          | 3.53        | 3.65        | 0.2     |
| Staphylococcus epidermidis      | >100        | >100        | >100    |
| Staphylococcus aureus           | 56.5        | 58.5        | 0.4     |
| Bacillus subtilis               | 14.1        | 14.6        | 0.4     |
| Bacillus cereus                 | 28.2        | 29.2        | 0.4     |

Structure determination revealed that compound 1 and compound 2 are tylosin analogues. Tylosin can inhibit bacterial growth by binding to the large ribosomal subunit to block the peptide tunnel [25]. Despite the structure difference at C6, compound 1 and compound 2 showed similar antibacterial activity, indicating that ethyl group at C6 position is replaceable with hydroxyl group without compromising the antibacterial activity (Fig. 5g). Compared to tylosin, the activity of compound 1 and compound 2 against most indicator strains was much lower. The reduction in activity of these compounds may be resulted from the absence of some active groups contributing to the tylosin activity, such as the aldehyde at C6 position, dimethyl amino as well as the saccharide moieties at C5 (Fig. 5c, f). No inhibitory activity against Staphylococcus epidermidis was observed with compound 1, compound 2 and tylosin at 100 μg/ml. However, very interestingly, compound 1 and compound 2 exhibited much higher activity against Streplococcus pneumoniae than tylosin (Table 2). S. pneumoniae strain with certain resistance to tylosin is probably due to the evolvement of pathogenic strains. Ribosome mutation is one way to obtain resistance to ribosome-targeted drugs. It was reported that replacing G2099 of ribosome with dimethyl adenine in Haloarcula marismortui triggered sterically clashing with dimethyl amino group linked to the saccharide moieties of tylosin and then the resistance was induced [26]. For compound 1 and compound 2, the reduced molecular size lacking dimethyl amino and saccharide branch at C5 could be beneficial for the compound to be accommodated into the ribosome tunnel of pathogenic strains. These results suggested that compound 1 and compound 2 could serve as starting molecules for further structural optimization to produce diverse bioactive agents, which are constantly required to combat the evolving pathogens and new diseases.

Conclusions

Two novel tylosin analogues were generated by Δwbla. Interestingly, the activity of compound 1 and compound 2 against S. pneumoniae was much higher than that of tylosin. They might serve as new derivatives of tylosin for property improvement by engineering combinatorial biosynthesis of metabolic pathways.
Methods
Strains, plasmids, primers and growth conditions
Strains and plasmids used in this study are listed in Table 3, and the primers used in this study are listed in Table 4. *Streptomyces ansochromogenes* 7100, a natural nikkomycin producer, and its derivatives were grown at 28 °C. SP medium (3 % mannitol, 1 % soluble starch, 0.75 % yeast extract and 0.5 % soy peptone, pH 6.0) was prepared for the production of antibiotics as described previously [27]. Agar minimal medium (MM) supplemented with mannitol as sole carbon source for sporulation was prepared [28]. *Escherichia coli* JM109, routinely used as a host for propagation of plasmids, was grown in Luria–Bertani (LB) medium at 37 °C. ET12567/pUZ8002 was used for conjugal transfer of DNA from *E. coli* to *Streptomyces* [28]. Tylosin tartrate was purchased from Sigma Aldrich, and used as a control in bioassays. All fungal strains used as indicators in this study except *C. albicans* were incubated for 5 days in PDA at 28 °C. *C. albicans* was grown in PDA for overnight at 37 °C.

Construction of recombinant strains
To construct the *wblA* disruption mutant (ΔwblA) of *S. ansochromogenes* 7100, the DNA fragment corresponding to the upstream region of *wblA* was amplified by PCR using primers LwblA-F and LwblA-R, and then it was digested with *Hind*III and *Xba*I. The pwblA1 was constructed by inserting above PCR product into the same sites of pKC1139. The DNA fragment corresponding to the downstream region of *wblA* was amplified by PCR using primers RwblA-F and RwblA-R, followed by digestion with *Bam*HI and *Eco*RV and inserted into the same sites of pwblA1 to generate pwblA2. Kanamycin resistance gene was amplified by PCR using primers Kan-F and Kan-R followed by digestion with *Bam*HI and *Xba*I, and inserted into the same sites of pwblA2 to generate pwblA3. Subsequently, pwblA3 was introduced into *S. ansochromogenes* 7100 via ET12567/pUZ8002 by conjugal transfer. The transformants resistant to kanamycin (Kanr) but sensitive to apramycin (Aprs) were selected and further confirmed by PCR using primers wblAJ-F and wblAJ-R. For complementation analysis, the fragment containing the intact *wblA* with its putative promoter region was amplified using primers CwblA-F and CwblA-R, and inserted into the *Eco*RV site of pSET152 to generate pSET152::wblA. Subsequently, pSET152::wblA was introduced into ΔwblA by conjugal transfer, and the resulting complemented strain was further confirmed by PCR. The null mutant was constructed by integrating pSET152 vector into the chromosome of ΔwblA as a control. All PCR amplicons were confirmed by sequencing.

Table 3 Strains and plasmids used in this study

| Name | Description | Sources |
|------|-------------|---------|
| Strains | | |
| *S. ansochromogenes* 7100 | Wild-type strain | [27] |
| ΔwblA | The ORF of *wblA* consists of 339 bp, and 230 bp of them was replaced by kanamycin resistance gene (neo) | This study |
| ΔwblA/pSET152::wblA | The complemented strain of ΔwblA | This study |
| *Escherichia coli* JM109 | recA1, endA1, gyrA96, thi-1, hsdR17, supE44, relA1, Δ(lac-proAB)/F’traD36, proAB + lacIq, lacZΔM15] | Invitrogen |
| *Escherichia coli* ET12567/pUZ8002 | *dam dcm hsdS cat tet* | [31] |
| *Staphylococcus aureus* CGMCC1.89 | Indicator strain for bioassays | CGMCC |
| *Bacillus subtilis* CGMCC1.1630 | Indicator strain for bioassays | CGMCC |
| *Bacillus cereus* CGMCC1.1626 | Indicator strain for bioassays | CGMCC |
| *Candida albicans* CGMCC2.4159 | Indicator strain for bioassays | CGMCC |
| *Alternaria longipes* CGMCC3.2946 | Indicator strain for bioassays | CGMCC |
| Plasmids | | |
| pwblA-DM | Plasmid used for the construction of ΔwblA | This study |
| pSET152::wblA | pSET152 containing the intact *wblA* with its putative promoter | This study |
| pSET152 | Integrative vector | [32] |
| pKC1139 | *E. coli-Streptomyces* shuttle vector | [28] |
| pBluescript KS+ | Routine cloning and subcloning vector | Stratagene |

CGMCC China General Microbiological Culture Collection Center
of 280 nm. Each experiment was performed in triplicate.

To 80% of methanol in water over 25 min at wavelength were eluted at 1 ml/min with a linear gradient from 50

For antibiotics production, spore suspensions were inoculated into liquid SP medium and cultured at 28 °C for

Isolation and structural determination of compound 1

Detection of nikkomycin and tylosin analogues

Figure S2. (A) Summary of key correlations between protons and carbons in compound 1. (B) 1H-1H COSY spectrum of compound 1. (C) 1H-13C HSQC spectrum of compound 1. (D) 1H-13C HMBC spectrum of compound 1.

Table S1. Antimicrobial activities of fermentation broth from S. ansochromogenes 7100 and ΔwblA by agar diffusion assays.

Additional file 1. Figure S1. NMR Spectra of compound 1. (A) Summary of key correlations between protons and carbons in compound 1 based on NMR spectroscopic data. (B) 1H-13C HSQC spectrum of compound 1. (C) 1H-13C HMBC spectrum of compound 1. (D) 1H-1H COSY spectrum of compound 1. (E) 1H-1H COSY spectrum of compound 2. (F) 1H-13C HSQC spectrum of compound 2. (G) 1H-13C HMBC spectrum of compound 2. Table S1. Antimicrobial activities of fermentation broth from S. ansochromogenes 7100 and Δwbla by agar diffusion assays.
Authors’ contributions
CL carried out experiments and analyzed the primary data. GL constructed the wblA mutant strain. JZ wrote and revised the manuscript. HT supervised the whole research work and revised the manuscript. All authors read and approved the final manuscript.

Author details
1 State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China. 2 University of Chinese Academy of Sciences, Beijing 100049, China. 3 College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China.

Acknowledgements
This work was supported by grants from the Ministry of Science and Technology of China (Grant nos. 2015CB150600 and 2013CB734001) and the National Natural Science Foundation of China (Grant nos. 31270110 and 31370097). We are grateful to Dr Zhoujie Xie, Professor Luyan Ma and Professor Baoshan Chen for kindly providing strains (Streptococcus pneumoniae 010, Streptococcus pyogenes #2, Staphylococcus epidermidis ATCC 35986, Pseudomonas aeruginosa PA14, Sporisorium scitamineum JG35 and Magnaporthe grisea Y34). We thank Drs Guomin Ai and Jinwei Ren (the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China) for assistance with Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) Spectroscopy.

Competing interests
The authors declare that they have no competing interests.

Received: 15 June 2015 Accepted: 8 October 2015
Published online: 02 November 2015

References
1. Du D, Wang L, Tian Y, Liu H, Tan H, Niu G. Genome engineering and direct cloning of antibiotic gene clusters via phage ΦBT1 integrate-mediated site-specific recombination in Streptomyces. Sci Rep. 2015;5:8740.
2. Li J, Li L, Feng C, Chen Y, Tan H. Novel polyoxins generated by heterologously expressing polyoxin biosynthetic gene cluster in the sanN inactivated mutant of Streptomyces ansochromogenes. Microb Cell Fact. 2012;11:35.
3. Feng C, Ling H, Du D, Zhang J, Niu G, Tan H. Novel nikkomycin analogues generated by mutasynthesis in Streptomyces ansochromogenes. Microb Cell Fact. 2014;13:59.
4. Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Butterfield NJ, Quail LA, et al. Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2). Nature. 2002;417:141–7.
5. Ohnishi Y, Ishikawa J, Hara H, Suzuki H, Ikeno Y, Ikeda H, Yamashita Y, Hattori M, Horinouchi S. Genome sequence of the streptomycin-producing microorganism Streptomyces coelicolor A3(2). J Bacteriol. 2002;184:3112–43.
6. Zhong X, Tian Y, Niu G, Tan H. Assembly and features of secondary metabolite biosynthetic gene clusters in Streptomyces ansochromogenes. J Bacteriol. 2008;190:4050–60.
7. Liu G, Chater KF, Chandra G, Niu G, Tan H. Molecular regulation of antibacterial biosynthesis in Streptomyces. Microbiol Mol Biol Rev. 2013;77:1124–43.
8. Chater KF. Regulation of sporulation in Streptomyces coelicolor A3(2): a checkpoint multiplex? Curr Opin Microbiol. 2001;4:667–73.
9. Fowler-Goldsworthy K, Gust B, Mouz S, Chandra G, Findlay KC, Chater KF. The actinobacteria-specific gene wblB controls major developmental transitions in Streptomyces coelicolor A3(2). Microbiology. 2011;157:1312–28.
10. Davis NK, Chater KF. The Streptomyces coelicolor wblABC gene encodes a small transcription factor-like protein dispensable for growth but essential for sporulation. Mol Gen Genet. 1992;232:351–8.
11. Noh JH, Kim SH, Lee HN, Lee SY, Kim ES. Isolation and genetic manipulation of the antibiotic down-regulatory gene, wblA ortholog for doxorubicin-producing Streptomyces strain improvement. Appl Microbiol Biotechnol. 2010;86:1145–53.
12. Nah JH, Park SH, Yoon HM, Choi SS, Lee CH, Kim ES. Identification and characterization of wblA-dependent tmcA regulation during tautomycin biosynthesis in Streptomyces sp Ck4412. Biotechnol Adv. 2012;30:202–9.
13. Rabyk M, Ostash B, Rebets Y, Walker S, Fedorenko V. Streptomyces grisea wblA ortholog for sporulation. Mol Gen Genet. 2013;290:1–8.
14. Fowler-Goldsworthy K, Gust B, Mouz S, Chandra G, Findlay KC, Chater KF. The actinobacteria-specific gene wblB controls major developmental transitions in Streptomyces coelicolor A3(2). Microbiology. 2011;157:1312–28.
15. Davis NK, Chater KF. The Streptomyces coelicolor wblABC gene encodes a small transcription factor-like protein dispensable for growth but essential for sporulation. Mol Gen Genet. 1992;232:351–8.
16. Noh JH, Kim SH, Lee HN, Lee SY, Kim ES. Isolation and genetic manipulation of the antibiotic down-regulatory gene, wblA ortholog for doxorubicin-producing Streptomyces strain improvement. Appl Microbiol Biotechnol. 2010;86:1145–53.
17. Nah JH, Park SH, Yoon HM, Choi SS, Lee CH, Kim ES. Identification and characterization of wblA-dependent tmcA regulation during tautomycin biosynthesis in Streptomyces sp Ck4412. Biotechnol Adv. 2012;30:202–9.
18. Rabyk M, Ostash B, Rebets Y, Walker S, Fedorenko V. Streptomyces grisea wblA ortholog for sporulation. Mol Gen Genet. 2013;290:1–8.
19. Fowler-Goldsworthy K, Gust B, Mouz S, Chandra G, Findlay KC, Chater KF. The actinobacteria-specific gene wblB controls major developmental transitions in Streptomyces coelicolor A3(2). Microbiology. 2011;157:1312–28.
20. Davis NK, Chater KF. The Streptomyces coelicolor wblABC gene encodes a small transcription factor-like protein dispensable for growth but essential for sporulation. Mol Gen Genet. 1992;232:351–8.
21. Noh JH, Kim SH, Lee HN, Lee SY, Kim ES. Isolation and genetic manipulation of the antibiotic down-regulatory gene, wblA ortholog for doxorubicin-producing Streptomyces strain improvement. Appl Microbiol Biotechnol. 2010;86:1145–53.