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Impact of *otrA* expression on morphological differentiation, actinorhodin production, and resistance to aminoglycosides in *Streptomyces coelicolor* M145*#

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Abstract: *otrA* resembles elongation factor G (EF-G) and is considered to be an oxytetracycline (OTC)-resistance determinant in *Streptomyces rimosus*. In order to determine whether *otrA* also conferred resistance to OTC and other aminoglycosides to *Streptomyces coelicolor*, the *otrA* gene from *S. rimosus* M527 was cloned under the control of the strong *ermE** promoter. The resulting plasmid, pIB139-*otrA*, was introduced into *S. coelicolor* M145 by intergeneric conjugation, yielding the recombinant strain *S. coelicolor* M145-OA. As expected *S. coelicolor* M145-OA exhibited higher resistance levels specifically to OTC and aminoglycosides gentamycin, hygromycin, streptomycin, and spectinomycin. However, unexpectedly, *S. coelicolor* M145-OA on solid medium showed an accelerated aerial mycelia formation, a precocious sporulation, and an enhanced actinorhodin (Act) production. Upon growth in 5-L fermentor, the amount of intra- and extracellular Act production was 6-fold and 2-fold higher, respectively, than that of the original strain. Consistently, reverse transcription polymerase chain reaction (RT-PCR) analysis revealed that the transcriptional level of pathway-specific regulatory gene *actII-orf4* was significantly enhanced in *S. coelicolor* M145-OA compared with in *S. coelicolor* M145.

Key words: *otrA* gene; *Streptomyces coelicolor*; Actinorhodin; Morphological differentiation; *actII-orf4*
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1 Introduction

Streptomyces species are Gram-positive soil bacteria known for their ability to produce a wide range of metabolites including clinically important antibiotics, biologically active compounds along with agents used in the agricultural, veterinary, and food industries (Chandra and Chater, 2014; Niu et al., 2016; Takano et al., 2016). Strain improvement is indispensable for the profitable commercialization of these natural products (Chen et al., 2010; Baltz, 2016). Generally, the production of antibiotics and other secondary metabolites is species-specific. Among the natural products formed by the strains, many have antibiotic activity and the producing strains should be resistant/insensitive to their own products (Chater et al., 2010). Self-resistance mechanisms include inactivation of antibiotics, efflux of antibiotics, and modification to the susceptible molecular target (Blair

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et al., 2015; Hu et al., 2016) or its replacement by an insensitive one. In most cases, the resistance genes are located within the biosynthetic gene cluster of particular secondary metabolites. Coordinate expression of resistance genes and biosynthesis genes is important to guarantee strong antibiotic production (Mak et al., 2014). Therefore, the over- or heterologous expression of the resistance gene has been considered as an interesting strategy to improve antibiotic production. For instance, the combined overexpression of three resistance genes *drrABC* present in the doxorubicin (DXR) biosynthetic gene cluster was achieved in *Streptomyces peucetius* ATCC 27952, leading to a significant enhancement of DXR production (Malla et al., 2010).

Streptomyces rimosus, a well-known oxytetracycline (OTC) producer, has been studied at the genetic level for many years (Petković et al., 2006; Pickens and Tang, 2010). OTC and aminoglycosides both bind to the aminoacyl site (A site) of 16S rRNA within the prokaryotic 30S ribosomal subunits and interfere with protein synthesis (Yu et al., 2012). Three resistance genes namely *otrA*, *otrB*, and *otrC* are known to be involved in the self-resistance mechanism. It was demonstrated that *otrB* encodes a membrane transporter protein and *otrC* is an adenosine triphosphate (ATP)-binding cassette. Together they constitute a transporter involved in OTC export (Petković et al., 2006; Pickens and Tang, 2010). The introduction of extra-copies of *otrB* or *otrC* in *S. rimosus* leads to an increased tolerance of the bacteria to OTC and to an enhanced OTC production (Chu et al., 2012; Guo et al., 2012; Yu et al., 2012). OTRA bears significant amino acid sequence homology to the translation elongation factor thermo unstable (EF-Tu) and translation elongation factor G (EF-G) and is considered to be a putative translation elongation factor with the OTC resistance function (Doyle et al., 1991; Cundliffe and Demain, 2010; Mak et al., 2014). However, the exact function of *otrA* remains to be elucidated.

In our earlier work, a resistance gene (GenBank accession number: KT291434.1) was isolated from the antagonistic strain *S. rimosus* M527 (Lu et al., 2016). Its amino acid sequence is identical to OTRA of *S. rimosus*. In this study we over-expressed *otrA* in the model strain *Streptomyces coelicolor* M145 that produced the blue-pigmented polyketide antibiotic actinorhodin (Act) (Lee et al., 2012). We investigated the influence of the over-expression of this gene on the resistance to OTC as well as to aminoglycoside and non-aminoglycoside antibiotics in this heterologous host. Doing so we noticed that *otrA* overexpression unexpectedly accelerated morphological development of *S. coelicolor* and had a positive impact on Act.

2 Materials and methods

2.1 Materials

Polymerase chain reaction (PCR) reagents, restriction enzymes, Miniprep kits, and Gel Extraction kits were purchased from TaKaRa Biotechnology Co., Ltd., Japan.

2.2 Bacteria strains and plasmids

The plasmids, primers (restriction sites were underlined), and strains used in this study are listed in Table 1. *Escherichia coli* JM109 was used as general host for plasmid construction and gene cloning. Methylation-deficient strain *E. coli* ET12567/pUZ8002 was used as the donor for plasmid transfer to *Streptomyces* (Kieser et al., 2000). Strain *S. rimosus* M527, which was isolated and deposited at the China Center for Type Culture Collection (CCTCC; M2013270), Wuhan, China, was used as the source of the *otrA* gene. Act producer *S. coelicolor* M145 was provided by Prof. Andreas BECHTHOLD (University of Freiburg, Freiburg, Germany).

All recombinant DNA techniques were performed as described by Sambrook and Russell (2001). Plasmid pIB139 was a gift from Prof. ZX DENG (Shanghai Jiao Tong University, Shanghai, China). (Wang et al., 2012; Xu et al., 2017). With *S. rimosus* M527 genomic DNA as a template, a 1992-bp *otrA* open reading frame (ORF) was amplified by PCR using primers P*otrA*-F/R (Table 1). The PCR product was then digested with *Nde*I and *Xba*I and inserted into the corresponding sites of pIB139, yielding plasmid pIB139-*otrA*. The inserted gene fragment was sequenced. The sequencing result confirmed that the gene did not contain any mutation. The control empty vector pIB139 and constructed pIB139-*otrA* harboring *otrA* controlled under *PermE** promoter were introduced by conjugation into parental strain *S. coelicolor* M145 and exconjugants were selected in the presence of apramycin at 50 μg/ml.

Table 1 Strains, plasmids, and primers used in this study

Strain, plasmid, or primer	Description	Source
Strain		
Streptomyces rimosus M527	*otrA* gene provider	Our lab
Streptomyces coelicolor M145	Actinorhodin producer	Prof. BECHTHOLD
Escherichia coli JM109	General cloning host	Our lab
E. coli ET12567 (pUZ8002)	Cm^r, Km^r, donor strain for conjugation	Our lab
S. coelicolor M145-OA	The *S. coelicolor* M145 strain harboring gene *otrA*	This work
Plasmid		
pIB139	Derivative of integrative plasmid pSET152, harboring a *PermE** promoter, apr^r, $oriT_{RK2}$, φC31 *int/attP*	Our lab
pIB139-*otrA*	*otrA* gene under the control of promoter *PermE** in plasmid pIB139	This work
Primer		
P*otrA*-F	5'-ACGCATATGATGAACAAGCTGAATCTGG-3' (*Nde*I)	This work
P*otrA*-R	5'-ACGTCTAGATCACACGCGCTTGAGCACG-3' (*Xba*I)	This work
PY*actII-orf4*-F	5'-ACGTCTAGACTACACGAGCACCTTCTCACCG-3'	This work
PY*actII-orf4*-R	5'-ACGCATATGATGAGATTCAACTTATTGGGAC-3'	This work
PY16S rDNA-F	5'-ACAAGCCCTGGAAACGG-3'	This work
PY16S rDNA-R	5'-AACAACCACTCCATCACCGAG-3'	This work

*Nde*I and *Xba*I restriction enzyme sites are underlined, respectively

2.3 Media and culture conditions

E. coli cells were grown in Luria-Bertani (LB) medium containing appropriate antibiotics. Antibiotics were used in the following final concentrations: apramycin (50 μg/ml), chloramphenicol (25 μg/ml), and kanamycin (50 μg/ml). To generate spores, *Streptomyces* cells were sprayed on a Mannitol-Soya flour (MS) medium and incubated for 6–7 d at 28 °C. *S. coelicolor* M145 and its derivative M145-OA were grown on a solid R2YE medium at 28 °C (Lee et al., 2012) for blue-pigmented Act production and phenotypic observation. CP medium was used as seed culture. CP liquid medium was composed of glucose 1% (10 g/L), yeast extract 0.4% (4 g/L), tryptone 0.2% (2 g/L), $MgSO_4·7H_2O$ 0.05% (0.5 g/L), K_2HPO_4 0.05% (0.5 g/L), NaCl 0.05% (0.5 g/L), and glycine 0.5% (5 g/L) (adjusted to pH 7.2 by NaOH).

S. coelicolor M145 spores ($1×10^6$ ml^{-1}) were inoculated into 40-ml CP liquid medium in a 250-ml Erlenmeyer flask and shaken at 28 °C and 180 r/min. Five percent of the seed culture was inoculated into 40 ml fermentation medium that contained 103 g sucrose, 10 g glucose, 5 g yeast extract, 0.1 g Difco casamino acids, 10.12 g $MgCl_2·6H_2O$, 0.25 g K_2SO_4, 5.73 g *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer (pH 7.2), 7 ml of 1 mol/L NaOH, and 1 ml of a trace element solution (composed of 80 mg $ZnCl_2$, 400 mg $FeCl_3·6H_2O$, 20 mg $CuCl_2·2H_2O$, 20 mg $MnCl_2·4H_2O$, 20 mg $Na_2B_4O_7·10H_2O$, and 10 mg $(NH_4)_6Mo_7O_{24}·4H_2O$) in 1 L of distilled water.

Fermentor (5 L; BIOTECH-5BG, Baoxing Biological Equipment Co., Shanghai, China) with a working volume of 3 L was used for the batch fermentation. The agitation speed and aeration rate were 200 r/min and 1.5 $m^3/(m^3·min)$, respectively. All assays were performed in triplicate. The reported values were then averaged (Xu et al., 2017).

2.4 Act production assays

R2YE medium was used to observe the production of the blue-pigmented antibiotic Act on agar media by directly evaluating the density of the blue color. To extract Act, culture samples (0.5 ml) were mixed with equal volumes of 2 mol/L KOH, vortexed, and centrifuged at 3000 r/min for 5 min. The Act concentration in the supernatant was determined by measuring the absorbance at 640 nm. The Act concentration was calculated based on an extinction coefficient of 25320 L/(mol·cm). Measurements were performed as described by Borodina et al. (2008) and Wang et al. (2008). Samples taken from bioreactors at different time points were analyzed for dry cell weight (DCW) and Act production.

2.5 Morphological observation of strain by scanning electron microscopy

The morphological characteristics of aerial hyphae of *S. coelicolor* M145 (parental strain) and recombinant M145-OA were observed after 4–5 d of incubation at 28 °C by scanning electron microscopy

(SEM; JSM-5410LV, JEOL, Tokyo, Japan) (Prakash and Nawani, 2014; Ma et al., 2016).

2.6 Determination of antibiotic resistance

In order to assess the resistance of strains, MS agar media containing different concentrations of antibiotics including OTC, rifampicin, gentamycin, hygromycin, ampicillin, and streptomycin were prepared. Minimum inhibitory concentration (MIC) was determined by spotting spore solutions (1×10^8) onto antibiotic-containing MS plates, followed by incubation for 3–4 d at 30 °C. The minimum drug concentration which fully inhibited growth was defined as the MIC (Ma et al., 2016).

2.7 Analysis of gene transcriptional levels by RT-PCR

Mycelia of *S. coelicolor* M145 and *S. coelicolor* M145-OA in the seed culture were collected at different time points and total RNA was extracted from the mycelial paste using Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol. To disrupt mycelia, the samples were immediately frozen in liquid nitrogen and ground under liquid nitrogen using a mortar and pestle. The RNA preparations were treated with RNase-free DNase I (TaKaRa, Japan) to eliminate possible chromosomal DNA contamination. The RNA concentration was determined by measuring absorbance at 260 nm (A_{260}) using a NanoDrop ND-2000 spectrophotometer (NanoDrop products, Wilmington, DE, USA). RNA purity was estimated by measuring the ratio of A_{260}/A_{280}, and an equal amount of RNA from each studied strain was used for the reverse transcription (RT) reaction. Complementary DNA (cDNA) first-strand synthesis was performed using PrimeScript™ RT reagent Kit (TaKaRa, Japan) according to the manufacturer's instructions. The amount of template used for RT-PCR and the number of PCR cycles for each gene were optimized in order to obtain enough visibility of the RT-PCR band and to ensure that amplification was in a linear range and that the results were semi-quantitative. For analysis of the pathway-specific regulatory gene *actII-orf4* involved in Act biosynthesis, resulting cDNA was used for PCR amplification under the following conditions: 5 min at 94 °C, followed by 30 cycles of 94 °C for 50 s, 58 °C for 30 s, and 72 °C for 30 s, and a final step at 72 °C for 10 min. 16S rDNA was used as an internal control to normalize the relative expression level of each target gene. The RT-PCR primers are listed in Table 1. The RT-PCR experiments were done in duplicate using RNA samples from two independent cultures.

2.8 Statistical analysis

All experiments were performed independently at least three times, and the mean values±standard deviations (SDs) were presented. The data were analyzed by Student's *t*-test. *P* values of <0.01 were considered statistically significant.

3 Results

3.1 Heterologous expression of *otrA* gene confers resistance to aminoglycosides to *S. coelicolor* M145

The gene *otrA* is considered as an OTC-resistance determinant in *S. rimosus* (Thaker et al., 2010). The N-terminal domain of OTRA bears similarities with the guanosine 5'-triphosphate (GTP)-binding sites of elongation factors, such as EF-G and EF-Tu (Chopra and Roberts, 2001). It is supposed to change the conformation of the 30S ribosome and in doing so prevents the binding of OTC to the latter, conferring resistance to OTC and to other aminoglycosides to the host. However, so far, this assumption is not confirmed. So in order to determine whether the over-expression of *otrA* also conferred enhanced resistance to aminoglycosides antibiotics to a heterologous host, *S. coelicolor*, the gene *otrA* of *S. rimosus* M527 was amplified by PCR using degenerate primers. The resulting PCR fragment was sequenced (GenBank accession number: KT291434.1) and cloned into the plasmid pIB139 carrying the apramycin-resistance (*apr*) gene. The obtained 1992-bp fragment encodes a 663-aa long protein identical to OTRA, a protein conferring resistance to tetracycline (NG048026.1, DQ143963.2), so the fragment was named gene *otrA*. In the plasmid the expression of *otrA* was placed under the control of $PermE^*$ promoter to yield pIB139-*otrA* (Fig. 1). The plasmid pIB139-*otrA* was then introduced into *S. coelicolor* M145 by intergeneric conjugative transfer between *E. coli*/*Streptomyces* to generate the recombinant strain *S. coelicolor* M145-OA resistant to 50 μg/ml

apramycin (Fig. S1). The integration of the *otrA* gene into the chromosome of *S. coelicolor* M145 was verified by amplification of the *otrA* gene from genomic DNA isolated from *S. coelicolor* M145-OA using the primers P*otrA*-F/R (Fig. S2). *S. coelicolor* M145-OA is significantly more resistant to OTC and also to gentamycin, hygromycin, streptomycin, and spectinomycin than the wild-type strain *S. coelicolor* M145, whereas both strains showed the same level of resistance to ampicillin and rifampicin (Table 2). In addition, there was no difference between *S. coelicolor* M145 and *S. coelicolor* M145 with empty plasmid pIB139 in terms of resistance to antibiotics (data not shown).

3.2 Effect of heterologous expression of *otrA* gene on the morphological development and Act production in *S. coelicolor* M145

Cell growth and morphological development of *S. coelicolor* M145-OA were observed throughout incubation. There was initially no significant difference between *S. coelicolor* M145-OA and *S. coelicolor* M145 up to 24-h cultivation, but beyond this point, *S. coelicolor* M145-OA showed an accelerated aerial mycelia formation (Fig. 2). SEM was employed to examine the differences of sporulation behavior between *S. coelicolor* M145-OA and *S. coelicolor* M145 (Fig. 3). More abundant sporulation in the strain *S. coelicolor* M145-OA (Fig. 3a) was visible than that of *S. coelicolor* M145 (Fig. 3b). In addition, there was no difference between *S. coelicolor* M145 and *S. coelicolor* M145 with empty plasmid pIB139 in terms of aerial mycelia and spores formation (data not shown).

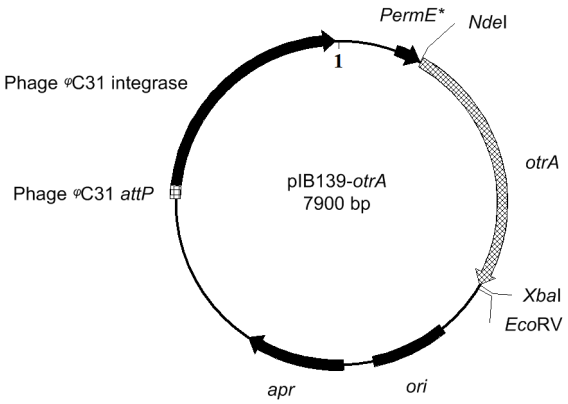

Fig. 1 Map of constructed plasmid pIB139-*otrA*

Table 2 Comparison of resistance levels to different antibiotics between *S. coelicolor* M145-OA and *S. coelicolor* M145

Strain	MIC (μg/ml)						
	Oxy	Rif	Gen	Hyg	Amp	Str	Spe
M145	100	80	50	100	>300	50	50
M145-OA	200	80	200	>200	>300	>100	>100

Spores (1×10^8) of *S. coelicolor* M145 and *S. coelicolor* M145-OA were prepared and plated on a set of MS agar plates containing different antibiotics with different concentrations. MS agar plates were incubated at 28 °C for 7 d. Oxy, oxytetracycline; Rif, rifampicin; Gen, gentamycin; Hyg, hygromycin; Amp, ampicillin; Str, streptomycin; Spe, spectinomycin

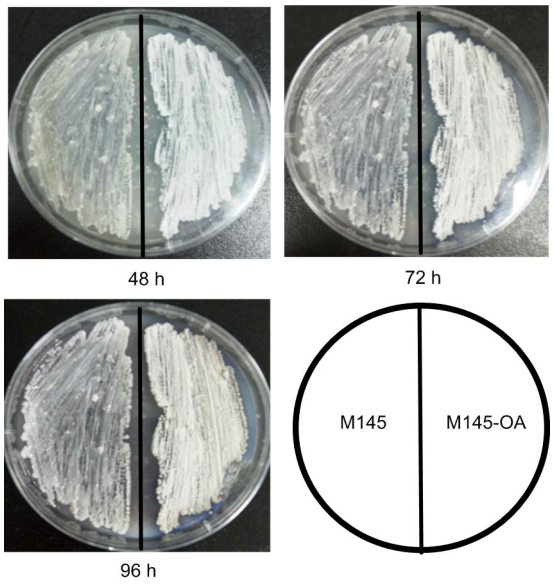

Fig. 2 Comparison of cell growth of *S. coelicolor* M145 and *S. coelicolor* M145-OA at different time intervals
The same amounts of spores (1×10^6) of *S. coelicolor* M145 and *S. coelicolor* M145-OA were streaked on MS agar media at 28 °C

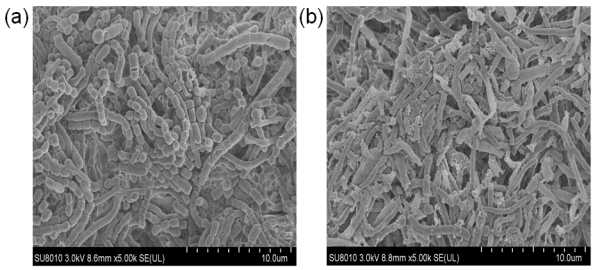

Fig. 3 Morphological characteristics of *S. coelicolor* M145 and *S. coelicolor* M145-OA
Scanning electron microscopy (SEM) analysis of aerial hyphae of *S. coelicolor* M145-OA (a) and *S. coelicolor* M145 (b)

To further investigate effects of *otrA* gene on Act production, four independent clones of *S. coelicolor* M145-OA were selected and analyzed. As shown in Fig. 4, *S. coelicolor* M145-OA produced more pigment associated with Act biosynthesis on R2YE agar medium than *S. coelicolor* M145. *S. coelicolor* M145-OA exhibited a darker pigment during the different cultivation time, suggesting that more Act was produced by this strain. To quantify Act more precisely, fermentation experiments were carried out in a 5-L stirred-vessel fermentor. As shown in Fig. 5, after 144-h fermentation, the intracellular amount of Act produced by *S. coelicolor* M145-OA was two-fold more than the amount produced by *S. coelicolor* M145. In addition, the amount of extracellular Act was also increased by 123.5%. The expression of empty plasmid pIB139 in *S. coelicolor* M145 has no effect on Act production. The *S. coelicolor* M145 with empty plasmid pIB139 produced the Act, the same as the *S. coelicolor* M145 did (data not shown).

The ActII-ORF4 protein plays an important and positive role in regulating the transcription of the genes involved in Act biosynthesis (Hesketh et al., 2001; Liu et al., 2017). To address the question whether overproduction of Act in M145-OA is exerted at the transcriptional level of regulator *actII-orf4*, we analyzed the expression levels of *actII-orf4* by RT-PCR in the wild-type *S. coelicolor* M145 and in its derivative strain *S. coelicolor* M145-OA. In agreement with the Act production, the recombinant strain M145-OA showed remarkably higher expression levels of *actII-orf4* compared with the corresponding value of wild-type strain *S. coelicolor* M145. It reached the highest level after 48 h in both *S. coelicolor* M145 and M145-OA (Fig. 6). As an internal control, heterologous expression of *otrA* had no effect on the expression of 16S rDNA.

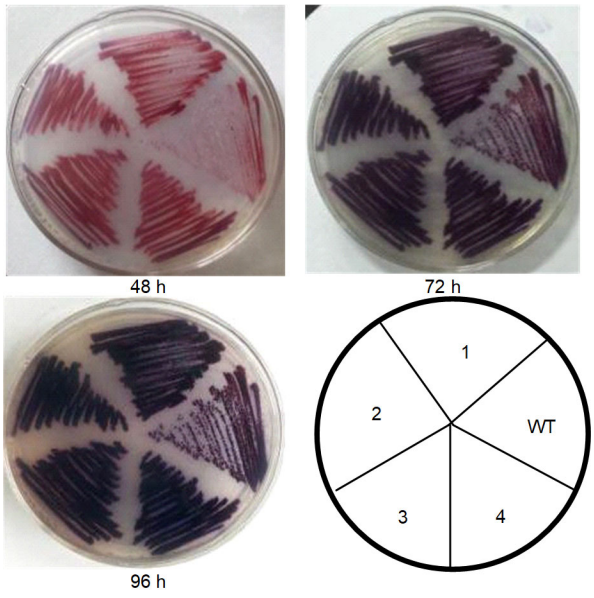

Fig. 4 Visual observation of Act production by *S. coelicolor* M145 and *S. coelicolor* M145-OA on the R2YE medium

Spores (1×10^8) were streaked on R2YE agar medium and then incubated at 28 °C for 4 d. The reverse sides of the plates are shown to indicate the amount of Act produced. Numbers 1–4: four randomly recombinant strains of *S. coelicolor* M145-OA; WT: *S. coelicolor* M145

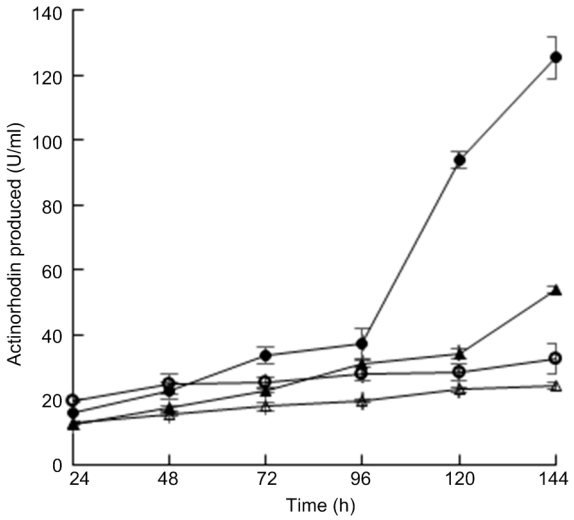

Fig. 5 Detection and comparison of Act concentration of wild-type *S. coelicolor* M145 (open) and recombinant strain *S. coelicolor* M145-OA (filled) in 5-L fermentor

Extracellular Act concentration: triangle; Intracellular Act concentration: circle. The error bars were calculated from three different batches of fermentation

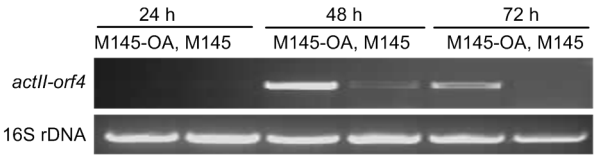

Fig. 6 Effect of heterologous expression of *otrA* gene on the transcriptional level of *actII-orf4* by using semi-quantitative reverse transcription-PCR analysis in the wild-type strain M145 and recombinant strain M145-OA

16S rDNA was used as the positive internal control. Cells were harvested from fermentation broth at 24, 48, and 72 h

Taken together, these findings indicated that heterologous expression of *otrA* affects not only morphological development but also the Act production in *S. coelicolor* M145.

4 Discussion

Self-resistance is required for survival of antibiotic producing streptomycetes. Numerous mechanisms conferring self-resistance to antibiotic producing strains have been identified (Piddock, 2006). Recent research has increasingly focused on the elucidation of the function of the proteins that confer resistance to antibiotics and the processes involved. A better understanding of the mechanism conferring resistance to the antibiotic in the producing strains may lead to the construction of a strain with enhanced ability to produce antibiotics. However, usually the over-expression of resistance mechanisms to a specific antibiotic correlates with the over-production of the cognate antibiotic. Unexpectedly, in this study we demonstrated the over-expression of a gene encoding a resistance protein OTRA (GenBank accession No. KT291434.1) conferred resistance to OTC and other aminoglycosides. It was reported that OTRA shows GTPase activity that is vital for the protection mechanism of the ribosome. OTRA confers OTC resistance by interacting with the ribosome and dislodging the bound OTC in a GTP-binding and hydrolysis-dependent manner (Mak et al., 2014). It has been described that the introduction of the *otrA* gene could enhance the host's resistance to OTC (Binnie et al., 1989; Doyle et al., 1991; Yin et al., 2017). In our study we showed that *S. coelicolor* M145-OA exhibited higher resistance to OTC than the control strain M145. In addition, resistance to aminoglycosides was also increased (Table 2). OTC and aminoglycosides both bind to the A site of 16S rRNA within the prokaryotic 30S ribosomal subunits and interfere with protein synthesis (Doi and Arakawa, 2007). Our results indicated that the ribosome protection site of OTRA includes the binding sites for both classes of antibiotics.

S. coelicolor M145 is a derivative strain of *S. coelicolor* A3(2). *S. coelicolor* A3(2) is one of the most studied actinomycetes, serving as a model strain used to investigate the molecular function of gene and its genome is the first one to be sequenced and annotated among the *Streptomycs* species. *S. coelicolor* M145 is generally able to produce the polyketide antibiotic Act (Coze et al., 2013), which serves as a model natural product for many scientists. Therefore, in this study, the effects of the heterologous expression of the *otrA* gene on morphological development and Act production were tested in *S. coelicolor* M145. Changes in morphological development and Act production indicated that OTRA might function as an elongation factor. Interestingly, in contrast to the 16S rRNA gene, the regulatory gene *actII-orf4* was much more strongly expressed under the influence of *otrA* indicating a more specific function towards the natural product biosynthesis of our protein. Our results are in accordance with the results of Yin et al. (2017), who reported that *otrA* over-expression rather than that of *otrB* and *otrC* in *S. rimosus* M4018 exhibits effects on OTC production. Surprisingly, over-expression of *otrA* in *S. rimosus* M527 had no effect on OTC production (data not shown), although it caused acceleration of morphological development (Fig. S3) and this result is similar to the results published by Chu et al. (2012), who described that expression of *otrA* in the industrial strain *S. rimosus* SRI had no effect on OTC production. At this moment we have no clear answer to these contradictory results. The different effects may be due to the different host strains.

The total amount of Act of M145-OA is increased significantly compared with the control strain M145. It is worth mentioning that the increase in the intracellular Act concentrations was higher than that in extracellular Act concentrations. One of the most likely possible factors contributing to the enhancement of Act production could be that expression of *otrA* increased the resistance to antibiotics in *S. coelicolor* M145.

5 Conclusions

The importance of this work is as follows: on the one hand, the accelerated morphological development and enhanced Act production caused by heterologous expression of the *otrA* gene in *S. coelicolor* M145 support the assumption that OTRA plays a role as a transcription factor as well as an elongation factor

involved in improving the translation process; on the other hand, it is confirmed that the increase of intracellular concentration of Act and promotion of resistance level to OTC as well as the aminoglycosides are due to protection by OTRA to the ribosome.

Contributors

Yan-fang ZHAO and Dan-dan LU participated in the design. Zheng MA wrote this article. Andreas BECHTHOLD revised the manuscript. Zheng MA and Xiao-ping YU checked the final version.

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Compliance with ethics guidelines

Yan-fang ZHAO, Dan-dan LU, Andreas BECHTHOLD, Zheng MA, and Xiao-ping YU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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List of electronic supplementary materials

Fig. S1 Phenotypic verification of seven randomly recombinant strains of *S. coelicolor* M145-OA

Fig. S2 PCR analysis of *otrA* gene from *S. coelicolor* M145-OA

Fig. S3 Morphological analyses of *S. rimosus* M527 and *S. rimosus* M527-OA on MS medium

中文概要

题　目：天蓝色链霉菌 M145 中 *otrA* 基因的表达对菌株形态分化、放线紫红素合成及氨基糖苷类抗生素抗性的影响

目　的：将来源于龟裂链霉菌（*Streptomyces rimosus*）M527 的 *otrA* 基因在天蓝色链霉菌 M145 中异源表达，通过考察宿主菌对土霉素以及氨基糖苷类抗生素的抗性、放线紫红素的合成、菌株形态等方面的变化鉴定 *otrA* 基因的功能。

创新点：*S. rimosus* 中的 *otrA* 基因与转录延伸因子 EF-G 具有很高的同源性，被认为是土霉素的抗性基因之一，但其具体的生物学功能目前尚未有研究报道。本文首次实现 *otrA* 基因在天蓝色链霉菌 M145 中的异源表达，不仅提高了宿主对土霉素以及氨基糖苷类抗生素的抗性，还促进了菌株产孢和放线紫红素的合成，从而初步证实了 OTRA 生物学功能。

方　法：克隆来源于 *S. rimosus* M527 的 *otrA* 基因，将其置于链霉菌整合型载体 pIB139 强启动子 *PermE** 的下游，构建重组质粒 pIB139-*otrA*；通过接合转移将其转入天蓝色链霉菌 M145 获得重组菌

M145-OA，实现 *otrA* 基因在天蓝色链霉菌 M145 中的异源表达；通过扫描电镜观察菌株的形态变化；通过含有不同浓度的不同抗生素的抗性平板筛选测试菌株的抗性水平变化；通过 5-L 发酵罐发酵实验考察次级代谢产物放线紫红素的合成能力变化；通过荧光定量 PCR 考察放线紫红素合成途径中的调控基因 *actII-orf4* 的转录水平变化。

结　论：来源于 *S. rimosus* M527 的 *otrA* 基因在天蓝色链霉菌 M145 中实现异源表达。一方面对宿主天蓝色链霉菌形态分化、放线紫红素产量的影响表明 *otrA* 可能作为一种类似延伸因子的发挥重要作用；另一方面宿主对土霉素及氨基糖苷类抗生素抗性的提高可能归因于OTRA对核糖体的保护作用。

关键词：*otrA* 基因；天蓝色链霉菌；放线紫红素；形态分化；*actII-orf4*