

Regulation of *myo*-inositol catabolism by a GntR-type repressor SCO6974 in *Streptomyces coelicolor*

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Abstract *Myo*-inositol is important for *Streptomyces* growth and morphological differentiation. Genomic sequence analysis revealed a *myo*-inositol catabolic gene cluster in *Streptomyces coelicolor*. Disruption of the corresponding genes in this cluster abolished the bacterial growth on *myo*-inositol as a single carbon source. The transcriptions of these genes were remarkably enhanced by addition of *myo*-inositol in minimal medium. A putative regulatory gene *SCO6974*, encoding a GntR family protein, is situated in the cluster. Disruption of *SCO6974* significantly enhanced the transcription of *myo*-inositol catabolic genes. *SCO6974* was shown to interact with the promoter regions of *myo*-inositol catabolic genes using electrophoretic mobility shift assays. DNase I footprinting assays demonstrated that *SCO6974* recognized a conserved palindromic sequence (A/T)TGT(A/C)N(G/T)(G/T)ACA(A/T). Base substitution of the conserved sequence completely abolished the binding of *SCO6974* to the targets demonstrating that *SCO6974* directly represses the transcriptions of *myo*-inositol catabolic genes. Furthermore, the disruption of *SCO6974* was correlated with a reduced sporulation of *S. coelicolor* in mannitol soya flour medium and with the overproduction of actinorhodin and calcium-dependent

antibiotic. The addition of *myo*-inositol suppressed the sporulation deficiency of the mutant, indicating that the effect could be related to a shortage in *myo*-inositol due to its enhanced catabolism in this strain. This enhanced *myo*-inositol catabolism likely yields dihydroxyacetone phosphate and acetyl-CoA that are indirect or direct precursors of the overproduced antibiotics.

Keywords *Streptomyces coelicolor* · *Myo*-inositol catabolism · *SCO6974* · Sporulation · Antibiotic production

Introduction

Streptomyces coelicolor is the soil-dwelling filamentous bacterium with a complex developmental life cycle. The life cycle begins with a spore, and it germinates and gives rise to the vegetative mycelium at presence of proper conditions and nutrients (Flårdh and Buttner 2009). In response to nutrient depletion, the vegetative mycelium grows from the colony surface into the air to form an aerial mycelium along with the onset of secondary metabolism (Chater 2011; Flårdh and Buttner 2009). Then, the aerial mycelium differentiates into chains of spores, which is an important event of morphological differentiation and tightly regulated by a complex regulation system such as *whi* genes (Chater 2011; McCormick and Flårdh 2012). The genome of *S. coelicolor* contains 29 secondary metabolite biosynthetic gene clusters. Four of them, responsible for the biosynthesis of the blue-pigmented polyketide antibiotic actinorhodin (ACT) (Malpartida and Hopwood 1984), the red oligopyrrole prodiginine antibiotics (RED) (Cerdeño et al. 2001), the lipopeptide calcium-dependent antibiotics (CDA) (Hojati et al. 2002), and the yellow-pigmented type I polyketide (yCPK) (Gottelt et al.

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2010), have been characterized. The regulation of secondary metabolism is a complex network (Liu et al. 2013), and the primary metabolites are the source of precursors for secondary metabolic biosynthesis. The onset of morphological differentiation and secondary metabolic biosynthesis is also affected by the limitation of phosphate (Sola-Landa et al. 2003; Martín 2004), nitrogen sources (Hesketh et al. 2002; Tiffert et al. 2008), glucose, or other easily utilized carbon sources (Borodina et al. 2008). The acetyl-CoA which is the initiator of tricarboxylic acid cycle is also used as the precursor of ACT biosynthesis (Ryu et al. 2006).

Myo-inositol is widely distributed in freshwater and soil; it can be used as carbon and energy source by many bacterial species (Bzymek et al. 2007; Turner et al. 2002). *Myo*-inositol catabolic pathway has been identified in many different bacteria, and the catabolic genes involved in the pathway are extensively conserved (Boutte et al. 2008; Yebra et al. 2007; Yoshida et al. 1997, 2008). These genes are generally clustered in bacterial genome, and usually a regulatory gene is situated in the cluster. In *Bacillus subtilis*, the *myo*-inositol catabolism is repressed by a DeoR family protein which binds to each of the *iol* and *iolRS* promoter regions (Yoshida et al. 1999). In *Sinorhizobium meliloti*, the regulator of *myo*-inositol catabolism belongs to the RpiR family, and it binds to the upstream regions of *idhA*, *iolY*, *iolR*, and *iolC* (Kohler et al. 2011). Both proteins recognize a conserved palindromic sequence, and the repression can be released by a *myo*-inositol catabolic intermediate (Kohler et al. 2010, 2011; Yoshida et al. 1999, 2008).

Myo-inositol is the precursor of phosphatidylinositol which is the crucial component of cellular membrane in *Streptomyces* (Chouayekh et al. 2007; Hoischen et al. 1997; Michell 2008). Inhibition of *myo*-inositol biosynthesis results in *myo*-inositol-dependent growth and development in *S. coelicolor* (Zhang et al. 2012). The mutant can grow at low concentration of *myo*-inositol, but cannot complete the morphological differentiation and is arrested at intermediate stages (Zhang et al. 2012). Thus, it is possible that the accumulation of *myo*-inositol and its derivatives is required for the sporulation septation of aerial hyphae in *S. coelicolor*.

Besides its biosynthesis, *myo*-inositol catabolism also affects the accumulation of *myo*-inositol in cells. However, there is no report about the *myo*-inositol catabolism and its regulation in *Streptomyces* as we know. In this study, the *myo*-inositol catabolic gene cluster was predicted in *S. coelicolor* based on the extensive studies of *myo*-inositol catabolism in other bacteria (Boutte et al. 2008; Yebra et al. 2007; Yoshida et al. 1997, 2008) and further verified by gene disruption. A cluster-situated regulatory gene *SCO6974* was identified and found to be required for the normal sporulation of *S. coelicolor*. Further studies demonstrated that the regulator *SCO6974* affected *Streptomyces* morphological differentiation by controlling the *myo*-inositol catabolism.

Materials and methods

Bacterial strains, plasmids, and growth conditions

Bacterial strains and plasmids used in this study are shown in Table S1. Mannitol soya flour (MS) medium or minimal medium (MM) supplemented with mannitol or *myo*-inositol as carbon source was used for the growth and sporulation of *S. coelicolor* M145 (SCP1⁻ SCP2⁻) and its derivatives, RNA preparation, as well as for the intergeneric conjugation of *S. coelicolor* (Kieser et al. 2000). R2YE medium was used for the production of actinorhodin (ACT) and undecylprodigiosin (RED). Difco Nutrient Agar (DNA) medium supplemented with 0.5 % NaCl (*w/v*) was used for the production of calcium-dependent antibiotics (CDA) (Kieser et al. 2000). Yeast extract-malt extract (YEME) liquid medium was used for the growth of *S. coelicolor* (Kieser et al. 2000). *Staphylococcus aureus* was used as the indicator of CDA production (Anderson et al. 2001). For detecting *myo*-inositol catabolism in *S. coelicolor*, the modified minimal medium (3.8 mM (NH₄)₂SO₄, 2.9 mM K₂HPO₄, 0.8 mM MgSO₄, 0.036 mM FeSO₄, and 27 mM *myo*-inositol) was used. *Escherichia coli* strains were cultured at 37 °C in Luria–Bertani (LB) medium supplemented with antibiotics (100 µg ml⁻¹ for ampicillin, 100 µg ml⁻¹ for kanamycin, 100 µg ml⁻¹ for apramycin, 100 µg ml⁻¹ for spectinomycin, 25 µg ml⁻¹ for chloramphenicol, and 15 µg ml⁻¹ for tetracycline) when necessary for propagating plasmids.

DNA manipulation, sequencing, and conjugation

Chromosomal DNA and plasmids were isolated from *Streptomyces* or *E. coli* according to the standard techniques (Kieser et al. 2000; Sambrook et al. 1989). DNA sequencing was performed by Biosune Company (Beijing, China). Database searching and sequence analysis were performed using the online program PSI-BLAST (Altschul et al. 1997). Plasmids or cosmids were firstly introduced by transformation into the methylation-deficient *E. coli* ET12567 (pUZ8002) and then transferred to *S. coelicolor* by intergeneric conjugation as described previously (Kieser et al. 2000).

Primers and PCR

All primers used in this study are listed in Table S2. The PCRs were carried out using *EasyTaq* DNA polymerase (TransGen Biotech), *EasyPfu* DNA polymerase (TransGen Biotech), KOD FX (TOYOBO), or KOD-Plus- (TOYOBO). An initial denaturation at 94 °C for 5 min was followed by 30 cycles of amplification (94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min), and additional 10 min at 72 °C (or 68 °C for KOD FX and KOD-plus-). Considering different DNA templates and

primers, the annealing temperature and the elongation time were changed in some cases.

Construction of the recombinant strains

Disruptions of the *myo*-inositol catabolic genes were based on the PCR targeting method with some modifications (Datsenko and Wanner 2000; Gust et al. 2003, 2004). A cosmid library of *S. coelicolor* M145 genomic DNA was constructed using SuperCos 1 vector kit (Stratagene) according to its protocol. Three cosmids containing the putative *myo*-inositol catabolic gene cluster *SCO2726*, *SCO6255*, and *SCO6974-6985* were isolated by screening the cosmid library with primers 2726YF1/2726YR1, 6255YF1/6255YR1, and 6984YF1/6984YR1, respectively. The plasmids pIJ773 and pIJ778 were used as the templates for PCR amplification of the cassettes *aac(3)IV-oriT* and *aadA-oriT* which were used to disrupt the target *myo*-inositol catabolic genes in these three cosmids. The amplified cassette was introduced into *E. coli* BW25113/pIJ790 carrying the target cosmids with corresponding *myo*-inositol catabolic genes by electroporation. The recombinants were obtained through the spectinomycin/apramycin resistance screening. The resulting mutagenized cosmids were transferred into *E. coli* ET12567/pUZ8002 and then introduced into *S. coelicolor* M145 by conjugal transformation. Subsequently, the corresponding *myo*-inositol catabolic gene disruption mutants were selected from exconjugants by spectinomycin (100 $\mu\text{g ml}^{-1}$) or apramycin (50 $\mu\text{g ml}^{-1}$) resistance and confirmed by PCR using primers listed in Table S2.

To construct the *SCO7254* disruption mutant, a DNA fragment corresponding to the upstream region of *SCO7254* was amplified by using primers 7254UF/UR. Then, the fragment was inserted into the *Hind*III site of pBluescript KS+::*Kan*^r, giving pD1. The other DNA fragment corresponding to the downstream region of *SCO7254* was amplified by using primers 7254DF/DR and ligated into the *Xba*I site of pD1 to give pD2. The DNA fragment was isolated with digesting the pD2 at *Hind*III-*Xba*I sites, and then it was inserted into the corresponding sites of pKC1132, giving pD7254. Subsequently, pD7254 was introduced into *E. coli* ET12567/pUZ8002 and then it was transferred to *S. coelicolor* M145 by intergeneric conjugation. After growing on MS agar supplemented with kanamycin (10 $\mu\text{g ml}^{-1}$) for 6 days at 28 °C, the colonies were transferred to MM medium with mannitol as the carbon source containing apramycin (10 $\mu\text{g ml}^{-1}$) or kanamycin (10 $\mu\text{g ml}^{-1}$). The kanamycin-resistant and apramycin-sensitive strains were selected and were confirmed by PCR using primers 7254YF1/YR1 and 7254YF2/YR2.

To construct the complemented strains of *SCO2726*, *SCO6975*, and *SCO6984*, these genes with their own promoters were amplified from genomic DNA using the corresponding primers 2726HF/HR, 6975HF/HR, and 6984HF/HR. Then, the amplified DNA fragments were digested with

*Xba*I/*Eco*RI and inserted into the same sites of pSET152 (Kieser et al. 2000). The resulting complemented plasmids were introduced into the corresponding disruption mutants by conjugation to generate the complemented strains. To construct the complemented and overexpressed strain of *SCO6974*, the entire *SCO6974* was amplified using the corresponding primers 6974HF/HR and inserted into the *Xba*I site of pSET152::P_{*rnnF*} (Pan et al. 2013). The resulting plasmid was introduced into the *SCO6974* disruption mutant and *S. coelicolor* M145 by conjugation.

RNA isolation and real-time RT-PCR

RNA was isolated from *S. coelicolor* M145 and the *SCO6974* disruption mutant (SCO6974DM) grown in MS or MM medium supplemented with or without *myo*-inositol for 24, 48, 72, 96, and 120 h as described previously (Liu et al. 2005). RNA was treated with DNase I (Promega) to remove the contaminated DNA, and then it was reverse-transcribed to complementary DNA by using PrimeScriptTM RT reagent kit (TaKaRa).

Real-time PCR was carried out in realplex² Master cycler (Eppendorf, Germany) using Ultra SYBR Mixture (CW BIO) with primers listed in Table S2. The conditions are used as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. The *hrdB* was used as an internal control. The relative transcriptional levels of tested genes were normalized to *hrdB* and determined by using the 2^{- $\Delta\Delta\text{CT}$} method (Livak and Schmittgen 2001). The values were presented as fold change in comparison with the relative expression levels for each gene at the first test time point in the wild-type strain. Data are presented as the averages of three independent experiments conducted in triplicate.

Heterologous expression and purification of SCO6974

The *SCO6974* coding region was amplified from genomic DNA using primers 6974OF and 6974OR. The amplified DNA fragment was digested with *Nde*I/*Xho*I and ligated into the corresponding sites of pET28a (Novagen) to generate the expressed plasmid pET28a:: *SCO6974*. After confirmed by DNA sequencing, the plasmid was introduced into *E. coli* C43 (Miroux and Walker 1996) for gene expression. *E. coli* C43 carrying pET28a:: *SCO6974* was cultured at 37 °C in 200 ml LB with 100 $\mu\text{g ml}^{-1}$ kanamycin to an OD₆₀₀ of 0.6. Isopropyl β -D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM, and the cultures were incubated for additional 3 h at 37 °C. After centrifugation (6000 \times g, 5 min, 4 °C), the cells were collected and washed with binding buffer (20 mM Tris-HCl, 500 mM NaCl, 5 mM imidazole, pH 7.9), and re-suspended in 20 ml of the same buffer. After sonication on ice, the cell suspension was centrifugated (12,000 \times g, 20 min, 4 °C) and the supernatant was recovered. After the supernatant was treated with Ni-NTA agarose

chromatography (Novagen), the purified His₆-SCO6974-His₆ was separated from the whole-cell lysate. The concentration of the purified proteins was determined using BCA protein assay reagent (Novagen). Protein purity was determined by Coomassie blue staining after SDS-polyacrylamide gel electrophoresis (PAGE) on a 10 % polyacrylamide gel. The purified protein was stored with 5 % glycerol at -70°C until used in the subsequent experiments.

Electrophoretic mobility shift assays

The electrophoretic mobility shift assays (EMSAs) were performed as described previously (Pan et al. 2009). DNA probes containing the promoter regions of *SCO2727*, *SCO6978-SCO6979*, and *SCO6985* were generated by PCR using the primers listed in the Table S2. The probe of *hrdB* was obtained by PCR using primers hrdBPF/hrdBPR and was used as negative control. EMSAs were carried out using DIG Gel Shift Kit, 2nd Generation (Roche) according to the manufacturer's instruction with some modifications. A mixture of the DIG-ddUTP labeled oligonucleotide probes and varying quantities of His₆-SCO6974-His₆ (0, 40, 80, 200, 400, and 2000 nM protein) was incubated at 25°C for 20 min in the reaction buffer which contained 1 μg of poly-(dI-dC) (Sigma), 20 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol (DTT), 10 mM MgCl₂, 54 mM KCl, 0.5 mg ml⁻¹ calf BSA, and 5 % glycerol in a total volume of 20 μl . Then, the mixture was separated on a 5 % native polyacrylamide gel with a running buffer containing 45 mM Tris-HCl (pH 8.0), 45 mM boric acid, and 1 mM EDTA at 150 V for 1 h. After electrophoresis, the DNA-protein complexes were blotted onto a nylon⁺ membrane by electroblotting, fixed by UV light, then blocked and washed. Finally, the shifted oligonucleotide was detected by anti-digoxigenin-AP conjugate and the chemiluminescent substrate CSPD[®] provided in the kit.

DNase I footprinting assays with fluorescence-labeled primers

DNase I footprinting assays were performed as described previously (Zianni et al. 2006). The forward primers were labeled with 6-carboxy fluorescein and the reverse primers were labeled with Hexachloro Fluorescein. The promoter regions of *SCO2727*, *SCO6978-SCO6979*, and *SCO6985* were amplified by primers used in EMSAs with labeling fluorescein. These probes were purified by using a Cycle-pure kit (Omega) and quantified with a NanoDrop 2000 (Thermo, USA). For this assay, 100 ng probes were incubated individually with varying quantities of His₆-SCO6974-His₆ in the same buffer as used in EMSAs in a total volume of 50 μl . After incubation for 20 min at 25°C , 1 μl of the solution containing 0.08 U of DNase I (Promega) and 5.5 μl of $10 \times$ DNase I reaction buffer [400 mM Tris-HCl (pH 8.0), 100 mM MgSO₄, 10 mM CaCl₂] were added, followed further

incubation at 37°C for 1 min. The reaction was stopped by adding 50 μl DNase I stop solution (20 mM EGTA, pH 8.0). Samples were extracted with phenol-chloroform and precipitated with ethanol, and the pellets were dissolved in 10 μl Milli-Q water. Then, the samples were sequenced by a company (Genolab, China), and the results were analyzed on GeneMarker V1.80 (<http://www.softgenetics.com>).

Alterations of the SCO6974-binding sequence

To evaluate the importance of the identified SCO6974 binding sites, the conserved sequence (A/T)TGT(A/C)N(G/T)(G/T)ACA(A/T) at the promoter region of *SCO2727*, *SCO6978-SCO6979*, and *SCO6985* were changed into (A/T)TAG(A/C)N(G/T) (G/T) GC T(A/T) using Easy Mutagenesis System (TransGen Biotech) with primers listed in Table S2. The binding capacity of His₆-SCO6974-His₆ to the mutagenized probes was measured by EMSAs as described above.

Mutational analysis of the SCO6978 binding sites of SCO6974

The wild-type and the mutagenized promoter regions of *SCO6978* were obtained using primers listed in Table S2. Then, the fragments were digested with *EcoRI/BamHI*, and inserted into the same sites of pSET152::*xylE* (Pan et al. 2009). The resulting plasmids were introduced into *S. coelicolor* M145 and the *SCO6974* disruption mutant by conjugation. These strains were cultured in MM medium supplemented with *myo*-inositol as carbon source for 24, 48, 72, 96, and 120 h. The activity of catechol dioxygenase in these strains was detected described previously (Pan et al. 2013).

Determination of antibiotic production

In order to assess the production of ACT and RED, 1×10^7 spores of the various strains were plated on cellophane discs laid on the surface of R2YE solid medium, and incubated at 28°C for 24, 48, 72, 96, 120, 144, and 168 h. Then, it was evaluated as described previously (Kieser et al. 2000). CDA production in DNA medium was assayed as described previously (Anderson et al. 2001).

Microscopy

The experiments of scanning electron microscopy were performed exactly as described previously (Pan et al. 2009).

Results

Identification of *myo*-inositol catabolic genes in *S. coelicolor*

Myo-inositol biosynthesis is important for the growth and morphological differentiation of *S. coelicolor* (Zhang et al. 2012), but little is known about its catabolism in *Streptomyces*. In search of the *S. coelicolor* genomic database, a whole set of putative *myo*-inositol catabolic genes were predicted based on the extensive studies of *myo*-inositol catabolic pathways in other bacteria (Boutte et al. 2008; Yebra et al. 2007; Yoshida et al. 1997, 2008). With the exception of *SCO2726* which encodes the putative methylmalonate semialdehyde dehydrogenase (homolog of *IolA*), the *myo*-inositol catabolic genes are situated in a single cluster (Fig. 1a). Three transcription units (*SCO6978-6974*, *SCO6979-6983*, and *SCO6985-6984*) were predicted in this cluster using an operon prediction program (www.microbesonline.org). Based on the prediction, *SCO6984* encodes a *myo*-inositol dehydrogenase (homolog of *IdhA*), *SCO6982* encodes a 2-keto-*myo*-inositol (2KMI) dehydratase (homolog of *IolE*), *SCO6975* encodes a

3-D-(3,5/4)-trihydroxycyclohexane-1,2-dione (THChDO) hydrolase (homolog of *IolD*), *SCO6976* encodes a 5-deoxy-D-glucuronic acid (5DG) isomerase (homolog of *IolB*), *SCO6978* encodes a 2-deoxy-5-keto-D-gluconic acid (DKG) kinase (homolog of *IolC*), *SCO6979-6981* encodes three sugar transport proteins, *SCO2727* and *SCO6977* encode the hypothetical proteins, *SCO6983* encodes a (4-hydroxy-3-nitro) benzylated polystyrene (PhnB)-like protein, and *SCO6985* encodes a phytanoyl-CoA dioxygenase. Besides the predicted *myo*-inositol catabolic genes, *SCO6255* and *SCO7254* also encode the paralogues of *myo*-inositol dehydrogenase. Thus, *myo*-inositol dehydrogenase encoded by *SCO6255*, *SCO6984*, and *SCO7254* may catalyze the first step of *myo*-inositol catabolism to form 2KMI. *SCO6982* is responsible for the second step to give THChDO which will be cleaved in the C2–C3 bond by *SCO6975* to yield 5DG. 5DG will be further catalyzed by *SCO6976*, *SCO6978*, and an unknown protein to form dihydroxyacetone phosphate (DHAP) and malonate semialdehyde (MSA) which will be degraded by *SCO2726* at the last step of *myo*-inositol catabolic pathway (Fig. 1b).

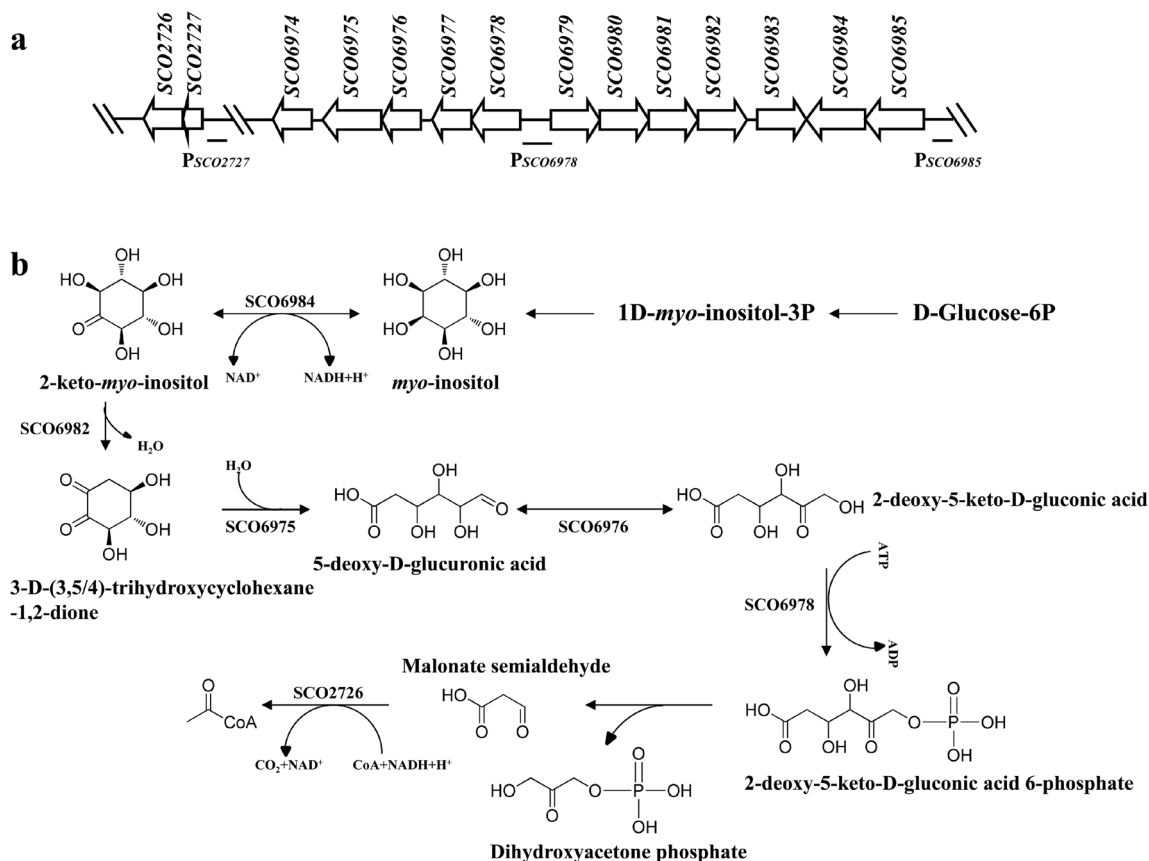


Fig. 1 Organization of the *myo*-inositol catabolic genes and the putative catabolic pathway of *myo*-inositol in *S. coelicolor*. **a** The *myo*-inositol catabolic genes are clustered and their coding regions are indicated by

arrows. **b** The putative catabolic pathway of *myo*-inositol in *S. coelicolor*. Functional assignments of the *myo*-inositol catabolic genes are based on the biochemical work in *B. subtilis* (Yoshida et al. 2008)

The catabolic genes are required for the bacterial growth on *myo*-inositol as a single carbon source

To confirm that these genes were responsible for *myo*-inositol catabolism in *S. coelicolor*, the following genes *SCO2726*, *SCO6255*, *SCO6975*, *SCO6976*, *SCO6978*, *SCO6982*, *SCO6984*, and *SCO7254* were disrupted and the mutants were confirmed by PCR (Fig. S1). When *myo*-inositol was used as the sole carbon source in the modified minimal medium as described in “Materials and methods” section, the disruption mutants of *SCO6975*, *SCO6976*, *SCO6978*, *SCO6982*, and *SCO6984* did not grow (Table 1), whereas the wild-type strain (WT) grew well. In the control experiment, all the strains grew well when glucose was used as the sole carbon source. Unexpectedly, the *SCO2726* disruption mutant still grew although slower than WT. However, the disruption mutants of *SCO6255* and *SCO7254* still grew well on *myo*-inositol as a single carbon source, suggesting that *SCO6255* and *SCO7254* are not essential for the *myo*-inositol catabolism in *S. coelicolor*. After complemented by the corresponding genes, the disruption mutant of *SCO2726*, *SCO6975*, and *SCO6984* restored to the normal growth on *myo*-inositol as a single carbon source (Table 1). Taken together, the gene cluster containing *SCO6975*, *SCO6976*, *SCO6978*, *SCO6982*,

and *SCO6984* is the main *myo*-inositol catabolic gene cluster in *S. coelicolor*.

Transcription of *myo*-inositol catabolic genes in *S. coelicolor*

Reverse transcriptase (RT) PCR confirmed that the *myo*-inositol catabolic gene cluster contains three transcription units which have been predicated by the operon prediction program (www.microbesonline.org) (Fig. S2). After addition of *myo*-inositol in the minimal medium with mannitol as the carbon source, the transcriptional level of *SCO2726*, *SCO6975*, *SCO6976*, *SCO6978*, *SCO6979*, *SCO6980*, *SCO6981*, *SCO6982*, and *SCO6984* were remarkably increased, especially the transcription of *SCO6984* increased 140-fold at the early stage (24 h) of growth (Fig. 2). However, the transcriptions of *SCO6255* and *SCO7254* (paralogues of *SCO6984*) did not show significant changes upon *myo*-inositol addition (Fig. 2). Considering that disruption of *SCO6255* or *SCO7254* did not affect the bacterial growth when *myo*-inositol was used as the single carbon source, our results clearly indicated that *SCO6984* is the only enzyme responsible for the first step of the *myo*-inositol catabolism.

SCO6974 is a conserved GntR protein and represses *myo*-inositol catabolism

A putative regulatory gene *SCO6974* was identified in the *myo*-inositol catabolic gene cluster (Fig. 1). The transcription of *SCO6974* in the minimal medium with mannitol as the carbon source almost remained the same level (Fig. 3), but it was clearly induced by *myo*-inositol (Fig. 2). The deduced protein *SCO6974* shows high similarity to the metabolite-responsive GntR family regulators which are widely distributed in prokaryotes (Hoskisson and Rigali 2009). Like most of GntR proteins, *SCO6974* contains a DNA-binding domain at its N-terminus and a ligand-binding domain at its C-terminus.

To investigate its function in *myo*-inositol catabolism, *SCO6974* was disrupted via PCR targeting system and the *SCO6974* disruption mutant was confirmed by PCR (Fig. S1). Disruption of *SCO6974* resulted in the transcriptional increase of the following *myo*-inositol catabolic genes: *SCO2726*, *SCO6975*, *SCO6976*, *SCO6978*, *SCO6979*, *SCO6980*, *SCO6981*, and *SCO6982* (Fig. 3), suggesting that *SCO6974* is a key repressor of *myo*-inositol catabolism. However, the transcriptional level of *SCO6984* responsible for the first step of *myo*-inositol catabolism did not increase in *SCO6974DM* (Fig. 3).

Repression of *myo*-inositol catabolism by *SCO6974* via direct protein-DNA interaction

To further understand the regulatory role of *SCO6974* in transcription of the *myo*-inositol catabolic genes, EMSAs were

Table 1 The catabolic genes are required for the bacterial growth on *myo*-inositol (MI) as the single carbon source

Strains	Growing status	
	MI as single carbon source	Glucose as single carbon source
WT (M145)	+	+
<i>SCO2726DM</i>	+/-	+
<i>SCO6255DM</i>	+	+
<i>SCO6975DM</i>	-	+
<i>SCO6976DM</i>	-	+
<i>SCO6978DM</i>	-	+
<i>SCO6982DM</i>	-	+
<i>SCO6984DM</i>	-	+
<i>SCO7254DM</i>	+	+
<i>SCO2726CM</i>	+	+
<i>SCO6975CM</i>	+	+
<i>SCO6984CM</i>	+	+

+ normal growth, +/-:slowly growth, -:no growth

WT the wild-type strain M145, *SCO2726DM* the *SCO2726* disruption mutant, *SCO6255DM* the *SCO6255* disruption mutant, *SCO6975DM* the *SCO6975* disruption mutant, *SCO6976DM* the *SCO6976* disruption mutant, *SCO6978DM* the *SCO6978* disruption mutant, *SCO6982DM* the *SCO6982* disruption mutant, *SCO6984DM* the *SCO6984* disruption mutant, *SCO7254DM* the *SCO7254* disruption mutant, *SCO2726CM* the *SCO2726* complemented strain, *SCO6975CM* the *SCO6975* complemented strain, *SCO6984CM* the *SCO6984* complemented strain

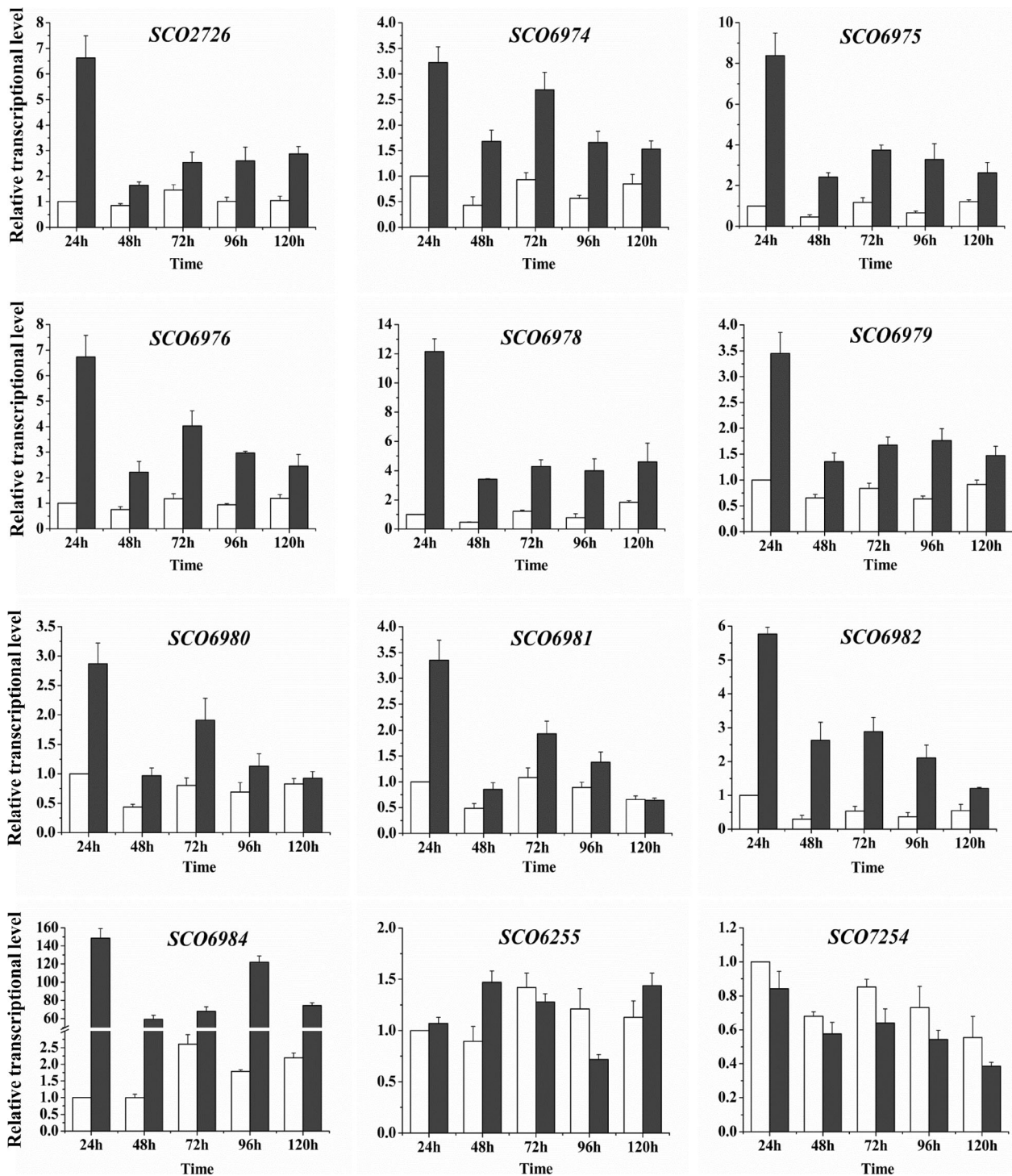


Fig. 2 Transcriptions of the catabolic genes are induced by *myo*-inositol. The transcriptional levels of *myo*-inositol catabolic genes were detected in WT by real-time RT-PCR after grown for 24, 48, 72, 96, and 120 h in MM medium with mannitol as the carbon source. All real-time RT-PCR data were normalized according to the abundance of *hrdB* in each sample. The

open columns represent the gene transcriptions without induction and the *solid columns* represent the gene transcriptions with induction of 5 mM *myo*-inositol. Data are presented as the averages of three independent experiments conducted in triplicate

performed. SCO6974 was overexpressed in *E. coli*, and the His₆-tagged SCO6974 was purified and used for EMSAs. Probes covering the promoter regions of SCO2727, SCO6978-SCO6979, and SCO6985 were used, respectively

(Fig. 1a). EMSAs demonstrated that SCO6974 (from 40 to 2000 nM) bound specifically to the SCO2727 promoter region where it formed three stable complexes and SCO6974 bound to the intergenic promoter region of SCO6978-SCO6979 to

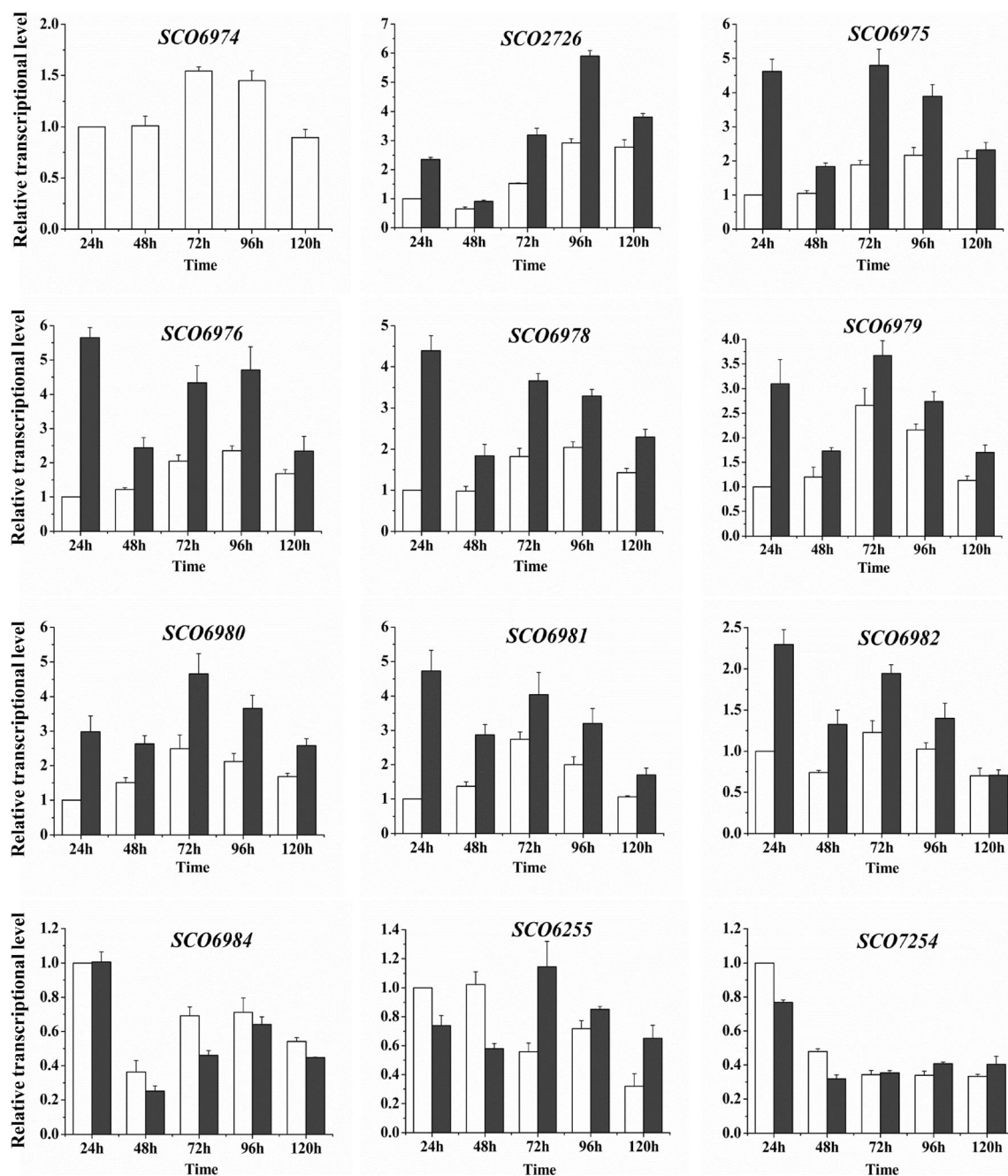


Fig. 3 Transcriptional analysis of the *myo*-inositol catabolic genes in WT and SCO6974DM. The transcriptional levels of *myo*-inositol catabolic genes were detected in WT or SCO6974DM by real-time RT-PCR after grown for 24, 48, 72, 96, and 120 h in MM medium with *myo*-inositol as the sole carbon source. All real-time RT-PCR data were normalized

according to the abundance of *hrdB* in each sample. The *open columns* represent the gene transcriptional levels in WT and the *solid columns* represent the gene transcriptional levels in SCO6974DM. Data are presented as the averages of three independent experiments conducted in triplicate

form four stable complexes and SCO6974 bound specifically to the *SCO6985* promoter region where it formed only one stable complex (Fig. 4). Specificity of binding was examined with the addition of 10- or 100-fold excess unlabelled probe, which abolished the binding ability of SCO6974 (200 nM) to the corresponding labeled fragments while addition of 100-fold excess unlabelled probe of *hrdB* could not abolish the binding ability (Fig. 4). These results revealed that

SCO6974 represses *myo*-inositol catabolism through interaction with the catabolic gene promoter regions directly.

To identify the specific binding sites of SCO6974, DNase I footprinting experiments were performed (Fig. 5). Protected regions were observed upstream of *SCO2727*, *SCO6978*–*SCO6979*, and *SCO6985*, respectively (Fig. 5). Sequence analysis showed that the SCO6974 binding sites is constituted by a palindromic sequence (A/T)TGT(A/C)

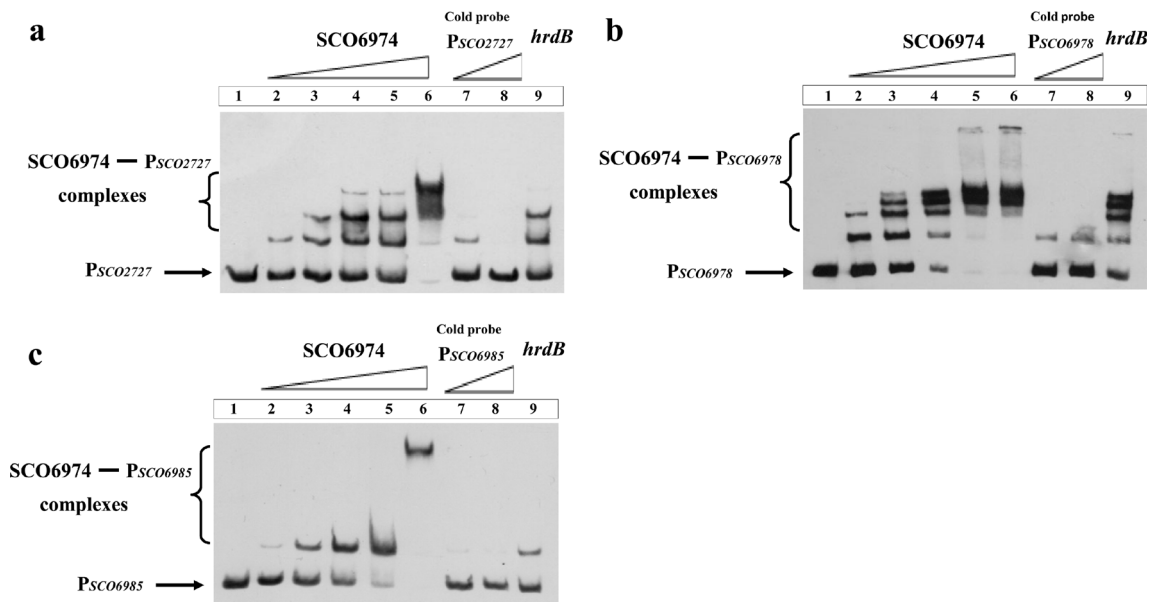


Fig. 4 EMSAs for detecting the binding of SCO6974 to the promoter regions of these catabolic genes. **a** Binding of SCO6974 to the promoter region of *SCO2727*. **b** Binding of SCO6974 to the intergenic promoter region between *SCO6978* and *SCO6979*. **c** Binding of SCO6974 to the promoter region of *SCO6985*. The probes containing the upstream regions of *myo*-inositol catabolic genes were incubated with increasing

amounts of His₆-SCO6974-His₆ (lanes 1–6 contain 0, 40, 80, 200, 400, and 2000 nM proteins, respectively). EMSAs of 200 nM His₆-SCO6974-His₆ with 10- and 100-fold excess of unlabelled specific probe are shown in lanes 7–8 and 100-fold excess of non-specific competitor *hrdB* are shown in lane 9. The arrows indicate the free probes and the braces show His₆-SCO6974-His₆-DNA complexes

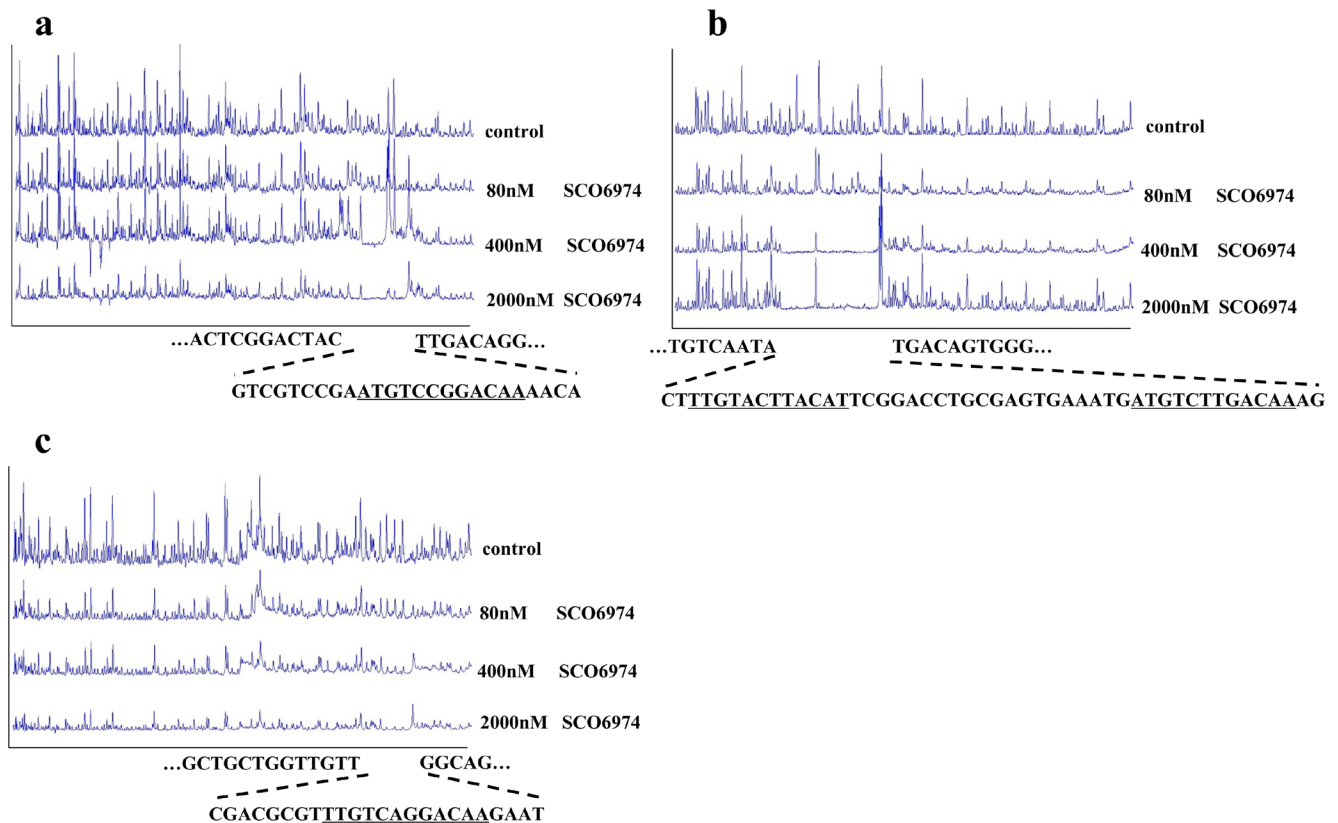


Fig. 5 Identification of the SCO6974 binding sites by DNase I footprinting assays in the promoter regions of *SCO2727* (**a**), *SCO6978*-*6979* (**b**), and *SCO6985* (**c**). The binding sequence of SCO6974 is

underlined. The control reaction is shown in the upper electropherogram in each panel

N(G/T)(G/T)ACA(A/T). To assess the importance of the identified SCO6974 binding sites, the conserved binding sequence of SCO6974 was mutagenized to (A/T)TAG(A/C)N(G/T)(G/T)GCT(A/T). The base substitution analysis demonstrated that the binding capacity of SCO6974 to the mutagenized probes was completely abolished in comparison with their corresponding wild-type targets (Fig. S3). These results implied that this conserved sequence is important for SCO6974 binding activity. To test whether the SCO6974-binding to the conserved sequence was essential for the transcriptional repression in vivo, the *xylE* reporter gene, encoding catechol 2,3-dioxygenase, was placed under the control of *SCO6978* promoter region with either the wild-type or the mutagenized SCO6974 binding sites. As shown in Fig. S3, the activity of XylE was detected in both strains. The activity of XylE was significantly increased when the SCO6974 binding sequence was mutagenized. The increment of *xylE* expression under the control of *SCO6978* promoter with the mutagenized binding site in M145 was in agreement with that of *xylE* expression under the control of *SCO6978* wild-type promoter in SCO6974DM. Thus, SCO6974 represses the transcription of *myo*-inositol catabolic genes through binding to the specific sequence in the promoter regions of these genes.

Disruption of *SCO6974* reduces the sporulation of *S. coelicolor*

Myo-inositol is the precursor of phosphatidylinositol, so its biosynthesis is required for the normal sporulation septation of aerial hyphae in *Streptomyces* (Zhang et al. 2012). Disruption of *SCO6974* significantly enhanced the transcription of *myo*-inositol catabolic genes likely leading to a reduction of the intracellular pool of *myo*-inositol that is necessary for sporulation septation. Indeed in MS medium, SCO6974DM grew normally, but produced few spores in comparison with WT and the complemented strain (SCO6974CM) (Fig. 6). However, SCO6974DM was almost restored to normal sporulation septation when *myo*-inositol was added to the MS medium (Fig. 6), demonstrating that the sporulation defect in SCO6974DM was due to a shortage of *myo*-inositol. SCO6974 is thus likely to regulate *Streptomyces* spore formation via its role in the control of the *myo*-inositol content of the cells.

Disruption of *SCO6974* enhances antibiotic production in *S. coelicolor*

As the morphological differentiation is closely associated with secondary metabolism and some regulators of morphological differentiation also affect antibiotic production in *Streptomyces* (Flärdh and Buttner 2009), the productions of ACT, RED, and CDA were determined in SCO6974DM. In contrast with the deficiency of sporulation septation in MS, the ACT production of SCO6974DM was significantly

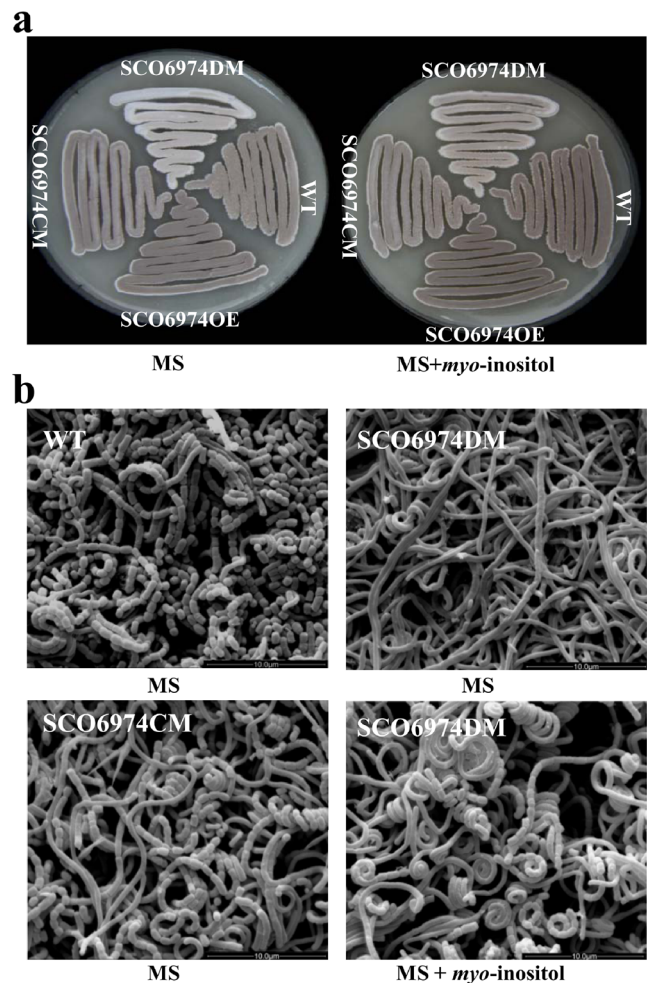


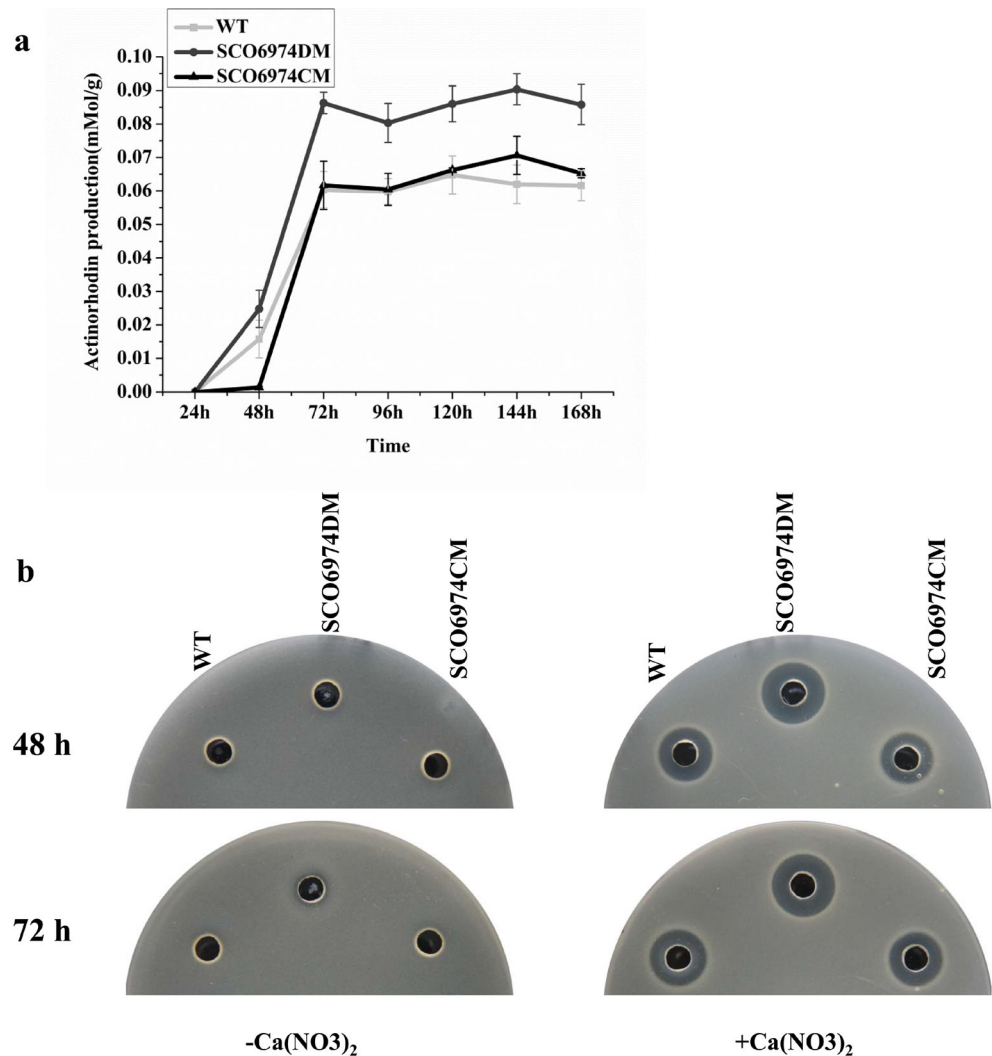
Fig. 6 Disruption of *SCO6974* impaired sporulation and the deficiency of sporulation in SCO6974DM was restored by addition of *myo*-inositol. **a** Morphological phenotype of the WT, SCO6974DM, SCO6974CM, and SCO6974OE in MS medium with or without addition of 40 mM *myo*-inositol for 72 h growth. **b** Scanning electron micrographs of the colony surfaces of WT, SCO6974DM, and SCO6974CM in MS medium, and SCO6974DM in MS medium with addition of 40 mM *myo*-inositol for 72 h growth

increased in R2YE medium compared with that of WT or SCO6974CM (Fig. 7a). SCO6974DM also produced more CDA in DNA medium than WT or SCO6974CM (Fig. 7b). However, there was no significant difference in the production of RED between SCO6974DM and WT (data not shown). It is possible that the enhanced *myo*-inositol catabolism yields DHAP and acetyl-CoA that are indirect or direct precursors of the overproduced antibiotics.

Discussion

In *S. coelicolor*, the biosynthesis of *myo*-inositol was recently found to be essential for the growth and morphological differentiation as the precursor of phosphatidylinositol which is the

Fig. 7 Disruption of *SCO6974* stimulated ACT and CDA production in *S. coelicolor*. **a** ACT production of WT, *SCO6974DM*, and *SCO6974CM* in R2YE medium. Cell cultures at each time point were treated with KOH (final concentration, 1 N) and the OD₆₄₀ corresponding to 1 g of mycelium was determined. **b** CDA production of WT, *SCO6974DM*, and *SCO6974CM* after grown for 48 and 72 h in DNA medium. *S. aureus* was used as the indicator strain in the presence or absence of Ca(NO₃)₂



crucial component of cell membrane (Zhang et al. 2012). However, a signaling/regulatory role for this molecule cannot be excluded. During sporulation septation of aerial hyphae, large amount of phosphatidylinositol and its derivatives were shown to be required (Zhang et al. 2012). Besides their structural role as constituent of membrane lipids, *myo*-inositol derivatives might have important signaling/regulatory functions necessitating a tight regulation of the *myo*-inositol levels. If *myo*-inositol is in limiting amount, the sporulation septation will be impaired but if *myo*-inositol exceeds bacterial needs, its degradation will be promoted. *inoR* is the repressor of *myo*-inositol biosynthesis in *S. coelicolor*, and the defect of *inoR* increases the amount of *myo*-inositol in vivo (Zhang et al. 2012). As expected, the transcriptions of *myo*-inositol catabolic genes were remarkably increased in the *inoR* mutant (Fig. S4). *SCO6974* may play a key role in the maintenance of *myo*-inositol balance during the morphological differentiation of *S. coelicolor* since it negatively controls the expression of most genes of the catabolic operons identified.

The transcriptions of genes in the cluster of *myo*-inositol catabolism were increased after the addition of *myo*-inositol (Fig. 2), and *SCO6974* repressed the transcriptions of most of the genes in the cluster (Fig. 3). Therefore, a regulatory mechanism might function in the presence of *myo*-inositol. *Myo*-inositol catabolism maintains a basal level in *S. coelicolor* due to its capable *myo*-inositol biosynthesis. While adding *myo*-inositol in medium, excess *myo*-inositol dissociates the repressor *SCO6974* from the promoter regions of *myo*-inositol catabolic genes indirectly, so the expressions of *myo*-inositol catabolic genes and *SCO6974* itself are increased which lead to the enhancing *myo*-inositol catabolism and consumption of *myo*-inositol. When *myo*-inositol is depleted, excess *SCO6974* binds to the promoters of *myo*-inositol catabolic genes again and *myo*-inositol catabolism returns to basal level.

Our results indicate that *SCO6984*, rather than *SCO6255* and *SCO7254*, is the real *myo*-inositol dehydrogenase catalyzing the first step of *myo*-inositol catabolism. However, its expression, that is strongly inducible by *myo*-inositol, is not

under the control of the GntR regulator SCO6974 that was shown to control negatively the expression of most of the other *myo*-inositol catabolic genes. This suggests that SCO6984 expression is also controlled by other regulators or the association of SCO6974 and other proteins. Considering SCO6984 is responsible for the first step of *myo*-inositol catabolism, it is easy to understand that expression of SCO6984 is highly controlled to keep the *myo*-inositol balance in *Streptomyces*. Our results demonstrated that the transcriptions of SCO6255 and SCO7254 were not induced by addition of *myo*-inositol in medium, and disruption of SCO6255 and SCO7254 did not inhibit *myo*-inositol catabolism in *S. coelicolor*. It will be interesting to know the function of SCO6255 and SCO7254 in future work. In *B. subtilis*, three inositol dehydrogenases (IolG, IolX, and IolW) have been found to catalyze different inositol stereoisomers (*myo*-, *D-chiro*-, and *scyllo*-inositols). IolG shows the catalytic activity to *myo*-inositol and *D-chiro*-inositol, IolX catalyzes *scyllo*-inositol, and IolW converts 2KMI to *scyllo*-inositol with NADPH oxidation (Morinaga et al. 2010). Combining the evidence that SCO6255, SCO6984, and SCO7254 are scattered in the different region of *S. coelicolor* genome, it is very possible that SCO6255, SCO6984, and SCO7254 play different roles in bacterial growth and differentiation, and they may have specific activity to the different inositol isomers.

The deduced protein SCO6974 shows high similarity to GntR family proteins. The metabolite-responsive GntR family proteins are widely distributed in bacteria and regulate various biological processes (Hoskisson and Rigali 2009). Among 57 putative GntR family regulators which respond to nutritional and/or environmental signals in *S. coelicolor*, DasR is well-known for its critical function in antibiotic production and morphological differentiation (Rigali et al. 2006). Like disruption of *dasR* resulted in the deficiency of aerial mycelium and spore formation, disruption of SCO6974 significantly reduced the sporulation septation of *S. coelicolor*. We examined the transcription of some developmental genes (*whiA*, *fisZ*, *whiB*, *crGA*, *ssgA*, *sigF*, *whiJ*, *whiD*, and *whiI*); however, except *ssgA*, other genes remained the same level between WT and SCO6974DM (Fig. S5). Addition of inositol did not affect the transcription of these genes (Fig. S5). It indicated that the few spores are produced by SCO6974DM mainly because of the shortage of *myo*-inositol.

Based on our results, disruption of SCO6974 enhanced the productions of ACT and CDA, suggesting that *myo*-inositol catabolism facilitates antibiotic production. Although *myo*-inositol is the structural basis for a number of secondary messengers in eukaryotic cells, it has not been proved the same in bacteria. As presence of many stereo- and regio-isomers of the inositol moiety and few of them has been studied, it could not exclude the possibility of inositol and its derivatives as signaling molecules during antibiotic production in *S. coelicolor*. As *myo*-inositol is a carbohydrate, its catabolites could also influence antibiotic

production through changing bacterial physiological development. As the production of *myo*-inositol catabolite acetyl-CoA is also the precursor of ACT, enhancement of *myo*-inositol catabolism will produce more acetyl-CoA and in turn increase ACT production. Disruption of SCO6974 also increased CDA production; it is speculated that *myo*-inositol metabolites or its derivatives stimulate CDA production either through the regulation or the precursors' accumulation.

In conclusion, the cluster of *myo*-inositol catabolism is identified in *S. coelicolor*, and SCO6974 directly regulates the catabolism of *myo*-inositol. SCO6974 also plays diverse roles in spore formation and the biosynthesis of antibiotics of *S. coelicolor*.

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Conflict of interest The authors declare that they have no conflict of interest.

References

- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389–3402. doi:10.1093/nar/25.17.3389
- Anderson TB, Brian P, Champness WC (2001) Genetic and transcriptional analysis of *absA*, an antibiotic gene cluster-linked two-component system that regulates multiple antibiotics in *Streptomyces coelicolor*. *Mol Microbiol* 39:553–566. doi:10.1046/j.1365-2958.2001.02240.x
- Borodina I, Siebring J, Zhang J, Smith CP, van Keulen G, Dijkhuizen L, Nielsen J (2008) Antibiotic overproduction in *Streptomyces coelicolor* A3(2) mediated by phosphofructokinase deletion. *J Biol Chem* 283:25186–25199. doi:10.1074/jbc.M803105200
- Boutte CC, Srinivasan BS, Flannick JA, Novak AF, Martens AT, Batzoglu S, Viollier PH, Crosson S (2008) Genetic and computational identification of a conserved bacterial metabolic module. *PLoS Genet* 4:e1000310. doi:10.1371/journal.pgen.1000310
- Bzymek KP, Newton GL, Ta P, Fahey RC (2007) Mycothiol import by *Mycobacterium smegmatis* and function as a resource for metabolic precursors and energy production. *J Bacteriol* 189:6796–6805. doi:10.1128/JB.00644-07
- Cerdeño AM, Bibb MJ, Challis GL (2001) Analysis of the prodiginine biosynthesis gene cluster of *Streptomyces coelicolor* A3(2): new mechanisms for chain initiation and termination in modular multienzymes. *Chem Biol* 8:817–829. doi:10.1016/S1074-5521(01)00054-0
- Chater KF (2011) Differentiation in *Streptomyces*: the properties and programming of diverse cell-types. In: Dyson P (ed) In *Streptomyces*. Molecular Biology and Biotechnology, Norfolk, pp 43–86
- Chouayekh H, Nothaft H, Delaunay S, Linder M, Payrastré B, Seghezzi N, Titgemeyer F, Viroille MJ (2007) Phosphoinositides are involved in control of the glucose-dependent growth resumption that follows

- the transition phase in *Streptomyces lividans*. J Bacteriol 189:741–749. doi:10.1128/JB.00891-06
- Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci USA 97:6640–6645. doi:10.1073/pnas.120163297
- Flärdh K, Buttner MJ (2009) *Streptomyces* morphogenetics: dissecting differentiation in a filamentous bacterium. Nat Rev Microbiol 7:36–49. doi:10.1038/nrmicro1968
- Gottelt M, Kol S, Gomez-Escribano JP, Bibb M, Takano E (2010) Deletion of a regulatory gene within the *cpk* gene cluster reveals novel antibacterial activity in *Streptomyces coelicolor* A3(2). Microbiol SGM 156:2343–2353. doi:10.1099/mic.0.038281-0
- Gust B, Challis GL, Fowler K, Kieser T, Chater KF (2003) PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. Proc Natl Acad Sci USA 100:1541–1546. doi:10.1073/pnas.0337542100
- Gust B, Chandra G, Jakimowicz D, Yuqing T, Bruton CJ, Chater KF (2004) Lambda red-mediated genetic manipulation of antibiotic-producing *Streptomyces*. Adv Appl Microbiol 54:107–128. doi:10.1016/S0065-2164(04)54004-2
- Hesketh A, Fink D, Gust B, Rexer HU, Scheel B, Chater K, Wohlleben W, Engels A (2002) The GlnD and GlnK homologues of *Streptomyces coelicolor* A3(2) are functionally dissimilar to their nitrogen regulatory system counterparts from enteric bacteria. Mol Microbiol 46:319–330. doi:10.1046/j.1365-2958.2002.03149.x
- Hoischen C, Gura K, Luge C, Gumpert J (1997) Lipid and fatty acid composition of cytoplasmic membranes from *Streptomyces hygroscopicus* and its stable protoplast-type L form. J Bacteriol 179:3430–3436, pmid: 9171384
- Hojati Z, Milne C, Harvey B, Gordon L, Borg M, Flett F, Wilkinson B, Sidebottom PJ, Rudd BA, Hayes MA, Smith CP, Micklefield J (2002) Structure, biosynthetic origin, and engineered biosynthesis of calcium-dependent antibiotics from *Streptomyces coelicolor*. Chem Biol 9:1175–1187. doi:10.1016/S1074-5521(02)00252-1
- Hoskisson PA, Rigali S (2009) Variation in form and function the helix-tumor-helix regulators of the GntR superfamily. Adv Appl Microbiol 69:1–22. doi:10.1016/S0065-2164(09)69001-8
- Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA (2000) Practical *Streptomyces* genetics. John Innes Centre, Norwich Research Park
- Kohler PR, Choong EL, Rossbach S (2011) The RpiR-like repressor IolR regulates inositol catabolism in *Sinorhizobium meliloti*. J Bacteriol 193:5155–5163. doi:10.1128/JB.05371-11
- Kohler PR, Zheng JY, Schoffers E, Rossbach S (2010) Inositol catabolism, a key pathway in *Sinorhizobium meliloti* for competitive host nodulation. Appl Environ Microbiol 76:7972–7980. doi:10.1128/AEM.01972-10
- Liu G, Chater KF, Chandra G, Niu G, Tan H (2013) Molecular regulation of antibiotic biosynthesis in *Streptomyces*. Microbiol Mol Biol Rev 77:112–143. doi:10.1128/MMBR.00054-12
- Liu G, Tian Y, Yang H, Tan H (2005) A pathway-specific transcriptional regulatory gene for nikkomycin biosynthesis in *Streptomyces anschrogenes* that also influences colony development. Mol Microbiol 55:1855–1866. doi:10.1111/j.1365-2958.2005.04512.x
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408. doi:10.1006/meth.2001.1262
- Malpartida F, Hopwood DA (1984) Molecular-cloning of the whole biosynthetic-pathway of a *Streptomyces* antibiotic and its expression in a heterologous host. Nature 309:462–462. doi:10.1038/309462a0
- Martín JF (2004) Phosphate control of the biosynthesis of antibiotics and other secondary metabolites is mediated by the PhoR-PhoP system: an unfinished story. J Bacteriol 186:5197–5201. doi:10.1128/JB.186.16.5197-5201.2004
- McCormick JR, Flärdh K (2012) Signals and regulators that govern *Streptomyces* development. FEMS Microbiol Rev 36:206–231. doi:10.1111/j.1574-6976.2011.00317.x
- Michell RH (2008) Inositol derivatives: evolution and functions. Nat Rev Mol Cell Biol 9:151–161. doi:10.1038/nrm2334
- Miroux B, Walker JE (1996) Over-production of proteins in *Escherichia coli*: mutant hosts that allow synthesis of some membrane proteins and globular proteins at high levels. J Mol Biol 260:289–298. doi:10.1006/jmbi.1996.0399
- Morinaga T, Ashida H, Yoshida KI (2010) Identification of two *scyllo*-inositol dehydrogenases in *Bacillus subtilis*. Microbiology 156:1538–1546. doi:10.1099/mic.0.037499-0
- Pan Y, Liu G, Yang H, Tian Y, Tan H (2009) The pleiotropic regulator AdpA-L directly controls the pathway-specific activator of nikkomycin biosynthesis in *Streptomyces anschrogenes*. Mol Microbiol 72:710–723. doi:10.1111/j.1365-2958.2009.06681.x
- Pan Y, Lu C, Dong H, Yu L, Liu G, Tan H (2013) Disruption of *rimP-SC*, encoding a ribosome assembly cofactor, markedly enhances the production of several antibiotics in *Streptomyces coelicolor*. Microb Cell Factories 12:65. doi:10.1186/1475-2859-12-65
- Rigali S, Nothaft H, Noens EE, Schlicht M, Colson S, Müller M, Joris B, Koerten HK, Hopwood DA, Titgemeyer F, Wezel GP (2006) The sugar phosphotransferase system of *Streptomyces coelicolor* is regulated by the GntR-family regulator DasR and links N-acetylglucosamine metabolism to the control of development. Mol Microbiol 61:1237–1251. doi:10.1111/j.1365-2958.2006.05319.x
- Ryu YG, Butler MJ, Chater KF, Lee KJ (2006) Engineering of primary carbohydrate metabolism for increased production of actinorhodin in *Streptomyces coelicolor*. Appl Environ Microbiol 72:7132–7139. doi:10.1128/AEM.01308-06
- Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, New York
- Sola-Landa A, Moura RS, Martín JF (2003) The two-component PhoR-PhoP system controls both primary metabolism and secondary metabolite biosynthesis in *Streptomyces lividans*. Proc Natl Acad Sci U S A 100:6133–6138. doi:10.1073/pnas.0931429100
- Tiffert Y, Supra P, Wurm R, Wohlleben W, Wagner R, Reuther J (2008) The *Streptomyces coelicolor* GlnR regulon: identification of new GlnR targets and evidence for a central role of GlnR in nitrogen metabolism in actinomycetes. Mol Microbiol 67:861–880. doi:10.1111/j.1365-2958.2007.06092.x
- Turner BL, Papházy MJ, Haygarth PM, McKelvie ID (2002) Inositol phosphates in the environment. Phil Trans R Soc London B 357:449–469. doi:10.1098/rstb.2001.0837
- Yebrá MJ, Zúñiga M, Beaufils S, Pérez-Martínez G, Deutscher J, Monedero V (2007) Identification of a gene cluster enabling *Lactobacillus casei* BL23 to utilize *myo*-inositol. Appl Environ Microbiol 73:3850–3858. doi:10.1128/AEM.00243-07
- Yoshida KI, Aoyama D, Ishio I, Shibayama T, Fujita Y (1997) Organization and transcription of the *myo*-inositol operon, *iol*, of *Bacillus subtilis*. J Bacteriol 179:4591–4598, pmid: 9226270
- Yoshida KI, Shibayama T, Aoyama D, Fujita Y (1999) Interaction of a repressor and its binding sites for regulation of the *Bacillus subtilis* *iol* divergon. J Mol Biol 285:917–929. doi:10.1006/jmbi.1998.2398
- Yoshida KI, Yamaguchi M, Morinaga T, Kinehara M, Ikeuchi M, Ashida H, Fujita Y (2008) *myo*-Inositol catabolism in *Bacillus subtilis*. J Biol Chem 283:10415–10424. doi:10.1074/jbc.M708043200
- Zhang G, Tian Y, Hu K, Zhu Y, Chater KF, Feng C, Liu L, Tan H (2012) Importance and regulation of inositol biosynthesis during growth and differentiation of *Streptomyces*. Mol Microbiol 83:1178–1194. doi:10.1111/j.1365-2958.2012.08000.x
- Zianni M, Tessanne K, Merighi M, Laguna R, Tabita FR (2006) Identification of the DNA bases of a DNase I footprint by the use of dye primer sequencing on an automated capillary DNA analysis instrument. J Biomol Tech 17:103–113, pmid: 16741237