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SCO3129, a TetR family regulator, is responsible for osmotic stress in *Streptomyces coelicolor*

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ABSTRACT

Streptomyces are the soil-dwelling bacteria with a complex lifecycle and a considerable ability to produce a variety of secondary metabolites. Osmoregulation is important for their lifecycle in nature. In the genome of *Streptomyces coelicolor* M145, *SCO3128* (encodes a putative fatty acid desaturase), *SCO3129* (encodes a putative TetR family regulator) and *SCO3130* (encodes a putative L-carnitine dehydratase) constitute a transcriptional unit, and its transcript was found to be in response to osmotic stress. Disruption of *SCO3130* led to a bald phenotype on MMG medium and the mycelia lysis on the edge of the colony when KCl/NaCl was added to the medium. These results indicated that SCO3130 is important for the osmotic stress resistance in *S. coelicolor*. Transcriptional analysis and electrophoretic mobility shift assays (EMSA) demonstrated that SCO3129 repressed the transcription of *SCO3128-3130* operon through directly binding to the promoter region of *SCO3128*, indicating that SCO3129 regulates the transcription of *SCO3128-3130* in response to osmotic stress.

1. Introduction

Members of the genus *Streptomyces* are well-known for their complex life cycle involving morphological differentiation and their ability to produce secondary metabolites [1]. In response to appropriate signals, generally believed, to include nutrient limitation, the substrate mycelium gives rise to aerial hyphae, which yield chains of spores to provide a mechanism for dispersal and the colonization of new environments [2].

Streptomyces have evolved an ability to grow in widely fluctuating environments in the soil or marine sediments. In their natural habitat, they are challenged with diverse nutritional and environmental stress [3], one of the most important and most frequent types of environment stress is changes in the external osmotic conditions. Thus, responding quickly and effectively to the constantly changing osmotic stress plays a key role in the survival of *Streptomyces* in natural environment. Regulatory proteins that respond to small molecule signals serve to activate or repress the transcription of genes that allow the organism to survive. The differentiation of *S. coelicolor* is an important strategy to respond to changes in the environment, especially nutrient starvation [4]. Evidence for coupling differentiation with stress-related signals has accumulated in recent years, and interplay of regulatory cascades with metabolic, morphological differentiation, social behavior, and stress-response has been proposed [5]. These include co-regulation of stress stimulons with developmental transitions participation of several stress-related sigma factors in initiating and completing differentiation process [6,7].

Our study describes an initial effort to illustrate the osmoregulation mechanism of a transcription unit *SCO3128-3130* in *S. coelicolor*. Our data demonstrated that K^+ could regulate the transcription of *SCO3128-3130* to cope with osmotic stress. The product of *SCO3129* is a DNA-binding protein that interacts directly with the *SCO3128-3130* promotor region to repress its transcription. This finding will provide a clue unravelling a signal transduction pathway from nutritional and osmotic stress to SCO3129 regulated gene expression in *S. coelicolor* and related bacteria, which is necessary for proper differentiation and/or stress survival.

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Table 1
Strains and plasmids used in this study.

Strains or plasmids	relevant characteristics	Source or reference
Strains		
S. coelicolor M145	Wild-type strain	[8]
SCO3128DM	The *SCO3128* disruption mutant	This work
SCO3129DM	The *SCO3129* disruption mutant	This work
SCO3130DM	The *SCO3130* disruption mutant	This work
SCO3130DM-C1	The SCO3130DM complement strain	This work
SCO3130DM-C2	The SCO3130DM complement strain	This work
E. coli DH5α	F- *recA* f80 d*lacZ* ΔM15	Gibco BRL
BL21(DE3)	F- *ompT hsdS gal dcm* (DE3)	Novagen
ET12567 (pUZ8002)	*recE dam dcm hsdS* Cm^r Str^r Tet^r Km^r	[30]
Plasmids		
pET28a	Expression vector	Novagen
pIMEP	*E. coli-Streptomyces* shuttle vector	[12]
pIMEP-3130	For construction of the complementary strain	This work
pKC1132	*E. coli-Streptomyces* shuttle vector	[8]
pET28a::*SCO3129*	*SCO3129* gene cloned in pET28a	This work
pUC119::*neo*	Provide the kanamycin resistance cassette (*neo*)	[9]
pIJ2925	Routine cloning and subcloning vector	[8]
pKC1132: *SCO3128*::*neo*	*SCO3128* disruption vector for *neo* inseration	This work
pKC1132: *SCO3130*::*neo*	*SCO3130* disruption vector for *neo* inseration	This work
pKC1132: *SCO3129*::*neo*	*SCO3129* disruption vector for *neo* inseration	This work
pKC1132: *SCO3*129LR	*SCO3129* marker-free deletion vector to replace *neo* with *SCO3*129LR	This work

Table 2
The primers used in this study.

Primers	Sequences (5′—3′)
Primers used for amplifying the neo gene	
KanF	ACGCGTCGACTCCCCTGGATACCGCTCG
KanR	TGAACTGCAGGAACCCCAGAGTCCCGC
Primers used for gene disruption	
3128DF	CCCAAGCTTCAGGTGGAAGTAGGTGGAGAAG
3128DR	ACGCGTCGACGTCTGGGATCACATCGGAACTCG
3129DF	TGCTGCTGTTCATCACCCTCCCGTTCC
3129DR	TGCACCGCCGTCATCACGACCTTCC
3130DF	CCCAAGCTTCAGTTCATCCTGTGGACGGTGGT
3130DR	CGGAATTCCCTCGCCCTCCAGGTCCCCG
3129DL01	CCCAAGCTTCTGTCCGACCGACTCCCAGGATA
3129DL02	CGGGGTACCGTCAATCATGCCGCCTTCGCCG
3129DR01	CGGGGTACCGCGACTCATAGGGCACCGCAAGA
3129DR02	CGGAATTCCCCGTCGGCAACCGCCTGGAGT
Primers used for confirming the read through of *SCO3128-3130*	
RT-F	TCCCAGACGCTCCGCAGCCCGC
RT-R	CCAGGTGCGTGCCCCGTCCCGT
Primers used for constructing the complemented strains	
C3129F	ATGATTGACGCCGTGGCGACCG
C3129R	CGGAATTCTCAGCTCTTGCGGTGCCCTATGAGTCG
C3130F	GCTCTAGAATGCAACCGCTGCGCGGAGTCAC
C3130R	GAAGATCTTCACACCACGCCGTCCGCGGC
Primers used for real-time RT-PCR	
R3127F	GCTTGGTGTTCTCCAGCTTC
R3127R	GGCTTCCTCTCCAACTCCAT
R3128F	CGCAAGCACCACAAGTTCTCCG
R3128R	GTCGAACATCCAGGCGATGTGG
RThrdB-F	CGCACCATCCGTATCCC
RThrdB-R	TCACCGAACTCGCTGTCG
Primers used for expression of SCO3129	
SCO3129F	GGGAATTCCATATGATTGACGCCGTGGCGACCG
SCO3129R	CCGCTCGAGGCTCTTGCGGTGCCCTATGAGTCG
Primers used for EMSAs	
3127EF	GGCGGTCTCCAGTTCGGTGC
3127ER	CGCGTCCTGGCCGACACCTA
3128EF	GCAGGCTCTGGGGCTCTTGC
3128ER	GCTGCGGAGCGTCTGGGATC

2. Materials and methods

2.1. Bacterial strains and growth conditions

The strains used in this study are listed in Table 1. *S. coelicolor* M145 and its derivatives were grown on MS (soya flour with mannitol) agar medium for conjugal transfer and MM [MMM (minimal medium contain 0.5% mannitol) or MMG (1% glucose as the carbon source)] for observing bacterial morphological differentiation. R2YE medium was used to detect the antibiotic production as described previously [8,9]. For DNA extraction, *Streptomyces* strains were grown in YEME liquid medium at 28 °C for 2 days. For routine subcloning, *Escherichia coli* DH5α and JM109 were grown at 37 °C in Luria–Bertani (LB) medium supplemented with ampicillin or apramycin when necessary. *E. coli* BL21(DE3) was used for protein expression [10,11]. The methylation-deficient *E. coli* ET12567 (*dam*$^-$*dcm*$^-$*hsds*$^-$) was used to propagate non-methylated DNA. *E. coli* ET12567 (pUZ8002) was used for conjugal transfer of DNA from *E. coli* to *Streptomyces*.

2.2. Plasmids construction and DNA transformation

The plasmid pIJ2925 was used for routine cloning and subcloning. Plasmids used in this study are listed in Table 1. The plasmid pKC1132 was used to construct the recombinant plasmids for gene disruption. The plasmid pUC119::*neo* was used as the template to amplify the kanamycin resistance gene *neo*. DNA manipulation, purification, ligation, restriction analysis, gel electrophoresis, and transformation of *E. coli* were carried out according to the standard methods [10]. Isolation of plasmid and genomic DNA, and transformation of *Streptomyces* were performed as described previously [8]. All primers used in this study are listed in Table 2.

The concentrations of antibiotics used in different media were as follows: for *E. coli*, kanamycin (100 μg/mL), ampicillin (100 μg/mL), apramycin (100 μg/mL) in LB; for *S. coelicolor*, apramycin (10 μg/mL), kanamycin (10 μg/mL) in solid MM medium, MS medium and liquid YEME medium. The stock concentrations of apramycin, ampicillin and apramycin were 100 mg/mL respectively.

2.3. Construction of the SCO3128 distruption mutant

To construct the *SCO3128* disruption mutant, a 2236 bp DNA fragment containing the entire *SCO3128* and its flanking sequence was amplified by PCR with the primers 3128DF/3128DR. It was then digested with *Hin*dIII-*sal*I and the DNA fragment containing *SCO3128* was ligated into the corresponding sites of pIJ2925 to generate p28DM-1. The *neo* gene was amplified by PCR with the primers KanF/KanR using pUC119::*neo* as the template and was inserted into the *Sma*I site of *SCO3128* in p28DM-1. The resulting plasmid was digested with *Hin*dIII-*Eco*RI and the 3232 bp DNA fragment was ligated into the corresponding sites of pKC1132 to give the plasmid pKC1132::*SCO3128*::*neo*. Then, the resulting plasmid was introduced into *S. coelicolor* M145 by conjugation via *E. coli* ET12567/pUZ8002. The kanamycin-resistance and apramycin-sensitive exconjugants were selected and the *SCO3128* disruption mutant was further confirmed by PCR using the primers 3128DF/3128DR.

2.4. Construction of the marker-less SCO3129 disruption mutant

A 2347 bp DNA fragment containing the entire *SCO3129* and its flanking sequence was amplified from M145 with the primers 3129DF/3129DR. It was then ligated into the *Hin*cII site of pIJ2925 to generate p29DM-1. The *neo* gene was amplified with the primers KanF/KanR by PCR using pUC119::*neo* as the template and was inserted into the *Hin*cII

site of *SCO3129* in p29DM-1. The 3343 bp *Hin*dIII/*Eco*RI fragment from p29DM-1 was ligated into the correspondent sites of pKC1132 to give the disruption plasmid pKC1132::SCO3129::*neo*. Then, the resulting plasmid was introduced into *S. coelicolor* M145 by conjugation via *E. coli* ET12567/pUZ8002. Kanamycin-resistance and aparmycin-sensitive exconjugants were selected as double-crossover disruption mutants.

To avoid the promoter of *neo* having polar effect on the transcription of *SCO3130*, *neo* was replaced by a second exchange. A 1205 bp DNA fragment containing the upstream region of *SCO3129* was amplified with the primers 3129DL01/3129DL02. It was digested with *Hin*dIII-*Kpn*I and then ligated into the correspondent sites of pIJ2925 to give the plasmid pIJ2925::*SCO3129DL*. The downstream region of *SCO3129* was amplified with the primers 3129DR01/3129DR02 and digested with *Kpn*I-*Eco*RI, and then ligated into the correspondent sites of pIJ2925::*SCO3129DL* to generate the plasmid pIJ2925::*SCO3129DL*::*SCO3129DR*. The 2507 bp *Hin*dIII/*Eco*RI fragment from pIJ2925::*SCO3129DL*::*SCO3129DR* was ligated into the correspondent sites of pKC1132 to give the disruption plasmid pKC1132::*SCO3129DL*::*SCO3129DR*. Then, the resulting plasmid was introduced into SCO3129DM by conjugation via *E. coli* ET12567/pUZ8002. The marker-free SCO3129DM was selected as kanamycin-sensitive and aparmycin-sensitive double-crossover mutant and was further confirmed by PCR with the primers 3129DF/3129DR.

2.5. Construction of the SCO3130 distruption mutant

A 3223 bp DNA fragment containing the entire *SCO3130* and its flanking sequence was amplified with primers 3130DF/3130DR. It was then digested with *Hin*dIII and *Eco*RI and ligated into the correspondent site of pIJ2925 to generate p30DM-1. The *neo* gene was amplified with the primers KanF/KanR by PCR using pUC119::*neo* as the template and was inserted into the *Apa*I site of *SCO3130* in p30DM-1. The 4219 bp *Hin*dIII-*Eco*RI fragment was isolated from p30DM-1 and ligated into the correspondent sites of pKC1132 to give the disruption plasmid pKC1132::SCO3130::*neo*. Then, the resulting plasmid was introduced into *S. coelicolor* M145 by conjugation via *E. coli* ET12567/pUZ8002. The kanamycin-resistance and aparmycin-sensitive exconjugant was selected as the double-crossover disruption mutant SCO3130DM and further confirmed by PCR with the primers 3130DF/3130DR.

2.6. Construction of the SCO3130 complemented strain

The *E. coli-Streptomyces* shuttle plasmid pIMEP (pSET152 with the *erm** promoter) was used to construct the complemented plasmid [12]. The entire open reading frame of *SCO3130* was amplified from M145 by PCR with the primers C3130F/C3130R and ligated into the *Eco*RV site of pIMEP to generate pIMEP-3130. Then, pIMEP-3130 was introduced into SCO3130DM by conjugation via *E. coli* ET12567/pUZ8002, and the apramycin-resistance exconjugants were selected.

2.7. Expression and purification of His_6-SCO3129

The entire *SCO3129* was amplified with the primers SCO3129F/SCO3129R. After digested with *Nde*I-*Xho*I, the amplified fragment was inserted into the corresponding sites of pET28a to generate a recombinant plasmid (pET28a::*SCO3129*), which was subsequently introduced into *E. coli* strain BL21(DE3) for *SCO3129* expression under the control of T7 promoter. The *E. coli* strain BL21 (DE3)/pET28a::*SCO3129* was grown at 37 °C overnight in LB medium supplemented with 100 μg/mL of kanamycin. One mL of the above overnight culture was inoculated into 200 mL of LB medium and incubated at 37 °C on a rotary shaker to an OD_{600} of 0.6. The culture was induced by 0.1 mM IPTG and incubated at 28 °C for additional 12 h. Cells harvested by centrifugation were washed twice with binding buffer [20 mM Tris-HCl, 500 mM NaCl, 5 mM imidazole, 5% glycerol (pH 7.9)] and then resuspended in 20 mL of the same buffer. After disruption of mild sonication, the cellular lysate was centrifuged at 12,000 g for 20 min and the soluble fractions were purified with Ni-NTA spin column (Qiagen) according to the manufacturer's manual. The concentration of the purified His_6-SCO3129 was determined by the method of Bradford using BSA as standard.

2.8. Electrophoretic mobility shift assays (EMSAs)

The EMSAs were performed as previously described with some modifications [11]. The probe P_{E27} containing a 159 bp upstream region of *SCO3127* was amplified with the primers 3127EF/3127ER. Similarly, the probe P_{E28} containing a 150 bp upstream region of *SCO3128* was amplified with the primers 3128EF/3128ER. During the EMSA, each DNA probe (5 ng) was incubated with various amounts of His_6-SCO3129 at 25 °C for 20 min in a total volume of 20 μL reaction mixture containing 20 mM Tris-HCl (pH7.9), 1 mM DTT, 5% glycerol, 0.04 μg/μL BSA and 10 mM $MgCl_2$. After incubation, the complexes and free DNA were separated by 4% non-denaturing polyacrylamide gels (mono/bis, 80:1) with a running buffer 0.5 × TBE containing 22.25 mM Tris-H_3BO_3 (pH 7.9), 20 mM sodium acetate and 1mM EDTA. Then the gel was stained by SYBR GOLD (Invitrogen).

2.9. Transcriptional analysis of SCO3127 and SCO3128

Total RNAs were isolated from strains grown in YEME medium as described previously [11]. Quality and quantity of RNAs were examined by UV spectroscopy and checked by agarose gel electrophoresis. To erase the chromosomal DNA contamination, each sample was treated with DNase I and tested by PCR to ensure that there was no chromosomal DNA left. After DNase treatment, RNA samples (1 μg) were reversely transcribed using SuperScript™ III and random hexamers (N15) as described by the vendor of the enzyme (Invitrogen). The probe P_{RT27} was amplified by the primers RT3127F/RT3127R; the probe P_{RT28} was amplified by the primers RT3128F/RT3128R. The relative level of amplified mRNA was normalized to mRNA expression of the housekeeping gene *hrdB*, which was amplified as an internal control using the primers RThrdB-F/RThrdB-R. Each reaction (20 μL) contained 0.1–10 ng of reversely-transcribed RNA depending on dilution, 10 μL *Power* SYBR Green PCR Master Mix (Applied Biosystems), 0.6 μM of both forward and reverse primers, respectively. The size of each amplicon is provided in parenthesis. The PCR reactive conditions were 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 96 °C for 30 s, 60 °C for 1 min, fluorescence was measured at the end of each cycle. Data analysis was made through Sequence Detection Software supplied by Applied Biosystems.

2.10. Scanning electron microscopy

For scanning electron microscopy, colonies were fixed in 2.5% (v/v) glutaraldehyde for 4 h, stained with osmic acid for 2–4 h and dehydrated with ethanol of different concentrations. Each specimen was coated with platinum–gold and then examined with a Hitachi D-570 scanning microscope.

3. Results

3.1. The transcription of SCO3128-3130 is induced by KCl

In the genome of *S. coelicolor* M145, *SCO3129* (encodes a putative TetR family regulator) and *SCO3130* (encodes a putative L-carnitine dehydratase) are located downstream of *SCO3128* (encodes a putative fatty acid desaturase) with the same transcriptional orientation. Gene organization and functional connection imply that these three genes constitute a co-transcriptional unit. It was confirmed by RT-PCR consequently (Fig. 1A).

The fatty acid desaturase can influence cell's membrane fluidity and

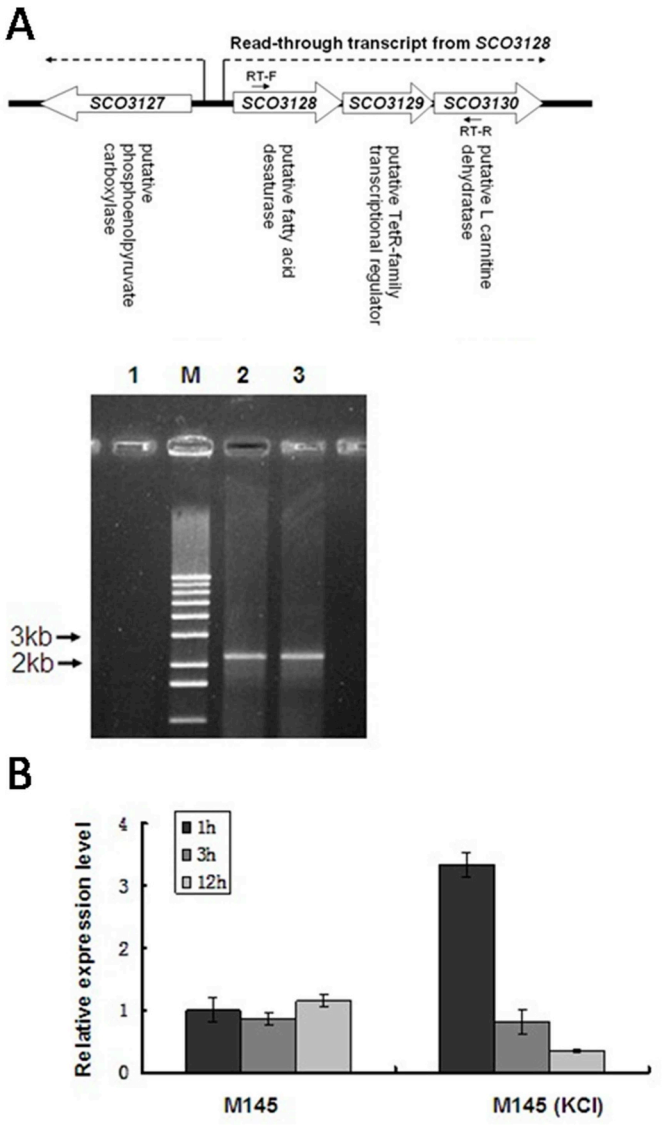

Fig. 1. Gene organization of *SCO3128-3130* and its transcription profile with or without KCl. (A) Gene organization of *SCO3128-3130* and RT-PCR analysis of the transcription of *SCO3128-3130* in *S. coelicolor* M145. The PCR templates were RNA (1, as negative control), genomic DNA (2, as positive control) and cDNA (3), respectively. (B) The transcription of *SCO3128* was induced by KCl. Strains were grown in YEME containing 10.3% sucrose for 15 h and treated with or without 1 M of KCl. RT-PCR analysis was done with the samples taken at 1 h, 3 h and 12 h after treated by KCl.

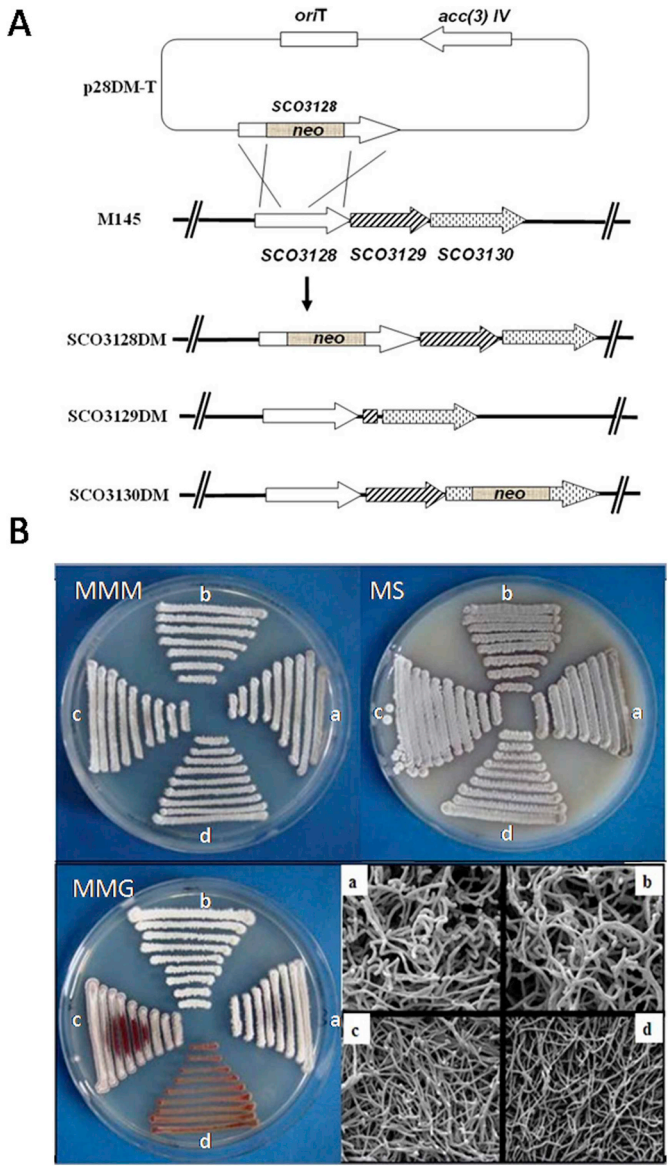

Fig. 2. Phenotype of strains SCO3128DM, SCO3129DM, SCO3130DM and M145 on MM agar. (A) Schematic representation of gene disruption. SCO3128DM, the *SCO3128* disruption mutant; SCO3129DM, the *SCO3129* disruption mutant; SCO3130DM, the *SCO3130* disruption mutant. (B) Phenotype of strains on MMM (mannitol as the sole carbon source), MS (soya flour with mannitol) and MMG (glucose as the sole carbon source). a, *S. coelicolor* M145; b, SCO3128DM; c, SCO3129DM; d, SCO3130DM. Scanning electron microscopy of SCO3128DM, SCO3129DM and SCO3130DM grown on MMG for 4 days.

increase the tolerance against hyperosmotic stress [6]. L-carnitine dehydratase catalyzes the conversion of L-carnitine to γ-butyrobetaine which is involved in the osmotic stress response in *E. coli* [13]. The *SCO3128-3130* was speculated to response to the osmotic stress in *S. coelicolor*. Based on the speculation, the transcriptional level of *SCO3128-3130* was detected when KCl was added in the medium as the osmotic agent. The real-time RT-PCR analysis revealed that the transcription of *SCO3128-3130* operon was induced by KCl rapidly. The maximum transcriptional level was found after 1 h induction and decreased gradually (Fig. 1B). This result indicated that *SCO3128-3130* is involved in the salt stress response in *S. coelicolor*.

3.2. The phenotypes and salt sensitivity of SCO3128DM, SCO3129DM and SCO3130DM

To address the *in vivo* function of these three genes, we constructed the *SCO3128*, *SCO3129* and *SCO3130* disruption mutants, respectively (Fig. 2A). For detecting phenotypes of these mutants, the media MMM, MMG, MS and R2YE were used. The *SCO3128* disruption mutant (SCO3128DM) did not show any obvious difference comparing with the wild-type M145. The *SCO3129* disruption mutant (SCO3129DM)

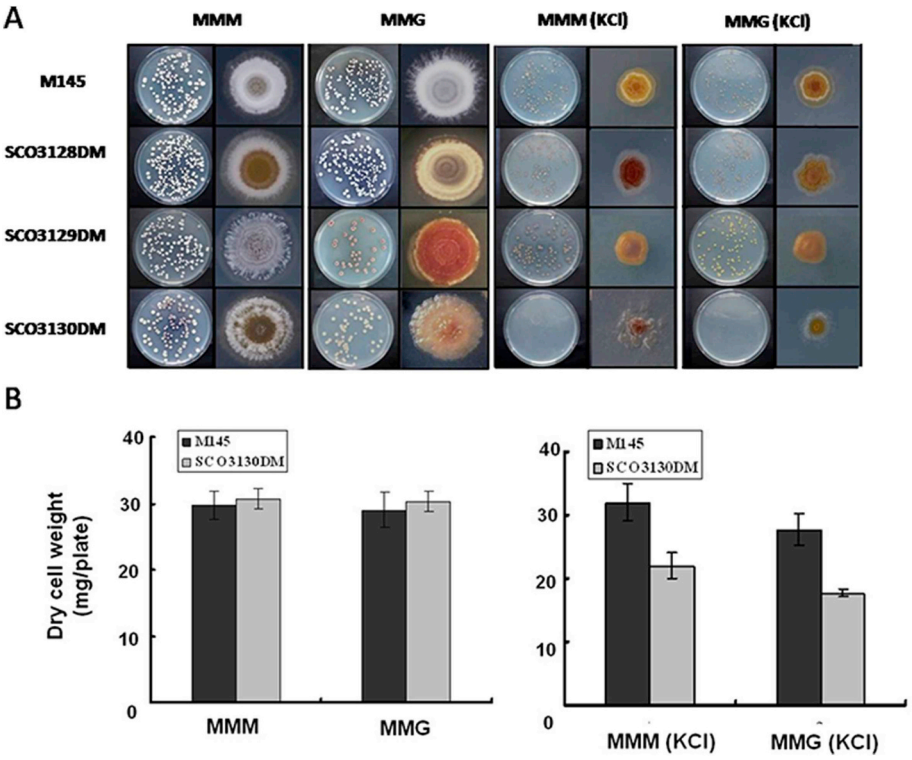

Fig. 3. Salt sensitivity analysis of each mutant. Salt sensitivity of SCO3128DM, SCO3129DM and SCO3130DM was examined by streaking cells on MM plates containing 1 M of KCl. A, Colony morphology of each mutant. Strains were grown at 28 °C for 5 days on MMM or MMG. B, Analysis of cell weight of SCO3130DM and M145 on MM agar with or without KCl. 1×10^7 pre-germinated spores of SCO3130DM and M145 were spread on cellophane membrane on MMM or MMG (containing 1 M of KCl) respectively. Mycelia were harvested after 5 days growth, and then the dry cell weight was measured. The data was obtained as average of three independent experiments.

showed that the formation of the aerial hyphae was delayed for 64 h when growing on MMG (Fig. S1), while the wild-type phenotype on MMM and MS. The *SCO3130* disruption mutant (SCO3130DM) showed a bald phenotype on MMG, but it restored the normal phenotype on MMM and MS (Fig. 2B). The colony morphology of these mutants was then checked by scanning electron microscope (Fig. 2B). SCO3130DM was more sensitive to high osmolarity (KCl/NaCl/$MgCl_2$) than M145 (Fig. 3A and Fig. S2). In the MMM or MMG supplemented KCl, SCO3130DM only formed very small colonies, and its mycelia mass was much lower than the wild-type strain (Fig. 3B). Under the dissecting microscope, the mycelia lysis on the edge of colony was observed, indicating that *SCO3130* is responsible for maintaining the cellular osmotic balance.

To clarify whether the observed phenotype were due to the deletion of *SCO3130*, a wild-type *SCO3130* gene controlled by the *erm** promoter on plasmid pIMEP was introduced into SCO3130DM and the transformant's phenotypes were restored to the wild-type (Fig. 4). The salt sensitivity and defective developmental phenotypes of SCO3130DM suggest that a proper activity level of SCO3130 is necessary for osmotic balance and morphological differentiation process in *S. coelicolor*.

3.3. SCO3129 represses the transcription of SCO3128-3130

Through sequence analysis, the N-terminal of deduced SCO3129 shows high similarity with the transcription regulators AcrR [14], QacR [15], TtgR [16], JadR2 [17], EnvR [18] and MtrR [19] of TetR family [20] (Fig. 5). AcrR is used as a chemical sensors to monitor osmotic stress change in many bacterial species [21]. When specific ligand binds to AcrR, it will be released from the DNA-binding site and initiate the transcription of response genes. In the *SCO3128-3130* operon, SCO3129 possibly plays the similar role. Transcriptional analysis showed that SCO3129 repressed the transcription of *SCO3128-3130* operon while it had no obvious effect on the transcription of *SCO3127* which encodes a putative phosphoenolpyruvate carboxylase (Fig. 6A). The transcription

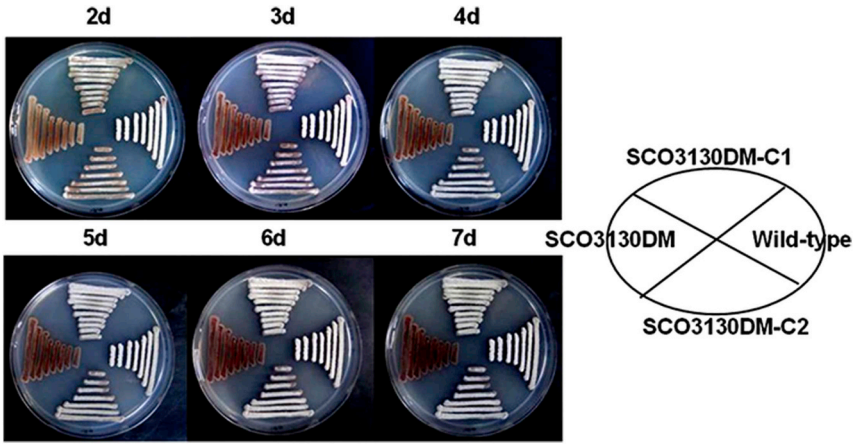

Fig. 4. Phenotypes of SCO3130DM and its complemented strain. SCO3130DM-C1 and SCO3130DM-C2 were random chosen as the complemented strains of SCO3130DM; Wild-type, the *S. coelicolor* M145. Phenotypes of these four strains were observed on MMG for 2–7 days.

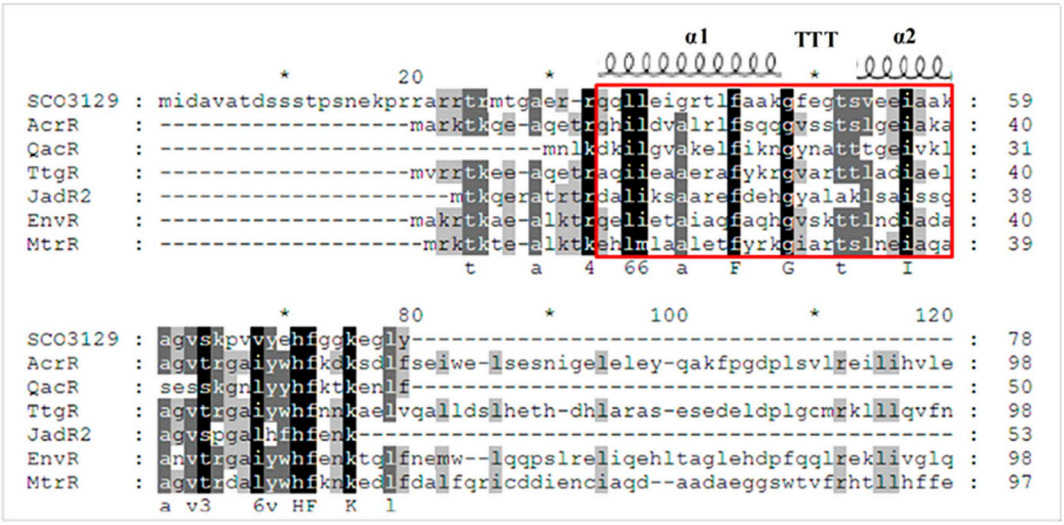

Fig. 5. Comparison of the deduced SCO3129 with other TetR family regulators. The boxes showed N-terminal conserved helix-turn-helix (HTH) DNA binding domain, a typical feature of TetR family regulators. Helix-turn-helix (HTH) designated as α1, TTT and α2. Dashes represent gaps introduced into the sequence to obtain the best consensus. Homologous regions of the SCO3129 polypeptides and the other five polypeptides are shaded in black or grey, respectively. The following sequences were used for comparison: AcrR (Accession no. NP752516.1); QacR (Accession no. NP115320.1); TtgR (Accession no. 743546.1); JadR2 (Accession no. AAB36583.1); EnvR (Accession no. NP627346.1); MtrR (Accession no. ZP05107326.1).

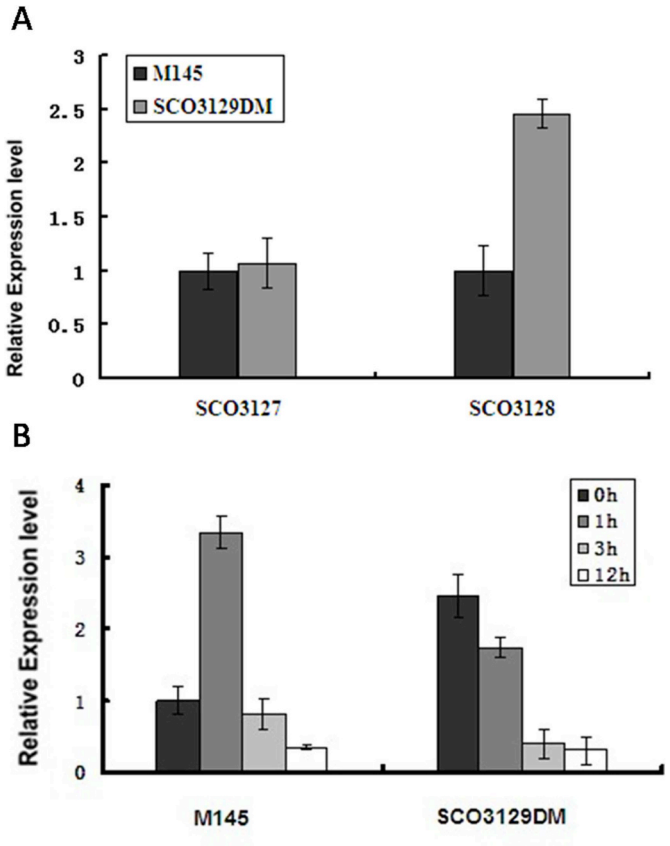

Fig. 6. Transcriptional analysis of *SCO3128* in M145 and SCO3129DM. A, The enhanced level of *SCO3128* and *SCO3127* transcripts in SCO3129DM. *S. coelicolor* M145 and SCO3129DM were cultured in YEME liquid medium containing 10.3% sucrose for 16 h. Total RNA was isolated and analyzed by realtime RT-PCR. B, Transcription level of *SCO3128* in M145 and SCO3129DM after treated with or without KCl for a serial times. Strains were grown in YEME containing 10.3% sucrose for 15 h and treated with or without 1 M of KCl. Sample harvested just before treated with KCl designated 0 h, then other three samples were taken at 1, 3 and 12 h, respectively, after treated with KCl.

of *SCO3128-3130* operon could not be induced by KCl in SCO3129DM although its transcriptional level increased (Fig. 6B). These results indicated SCO3129 is the sensor of osmotic stress during the transcription of *SCO3128-3130* operon.

3.4. SCO3129 binds to the promoter region of SCO3128-3130

To determine the detail role of SCO3129, EMSAs were carried out. *SCO3129* was overexpressed in *E. coli* and the His_6-tagged SCO3129 was purified to near homogeneity by a single chromatography on Ni-NTA resin (Fig. 7A). The DNA fragment P_{ET28} covering the promoter region of *SCO3128* and the DNA fragment P_{ET27} covering the promoter region of *SCO3127* were used as probes. EMSAs showed that the purified His_6-tagged SCO3129 could specifically bind to the promoter region of *SCO3128* (Fig. 7B), but not to P_{ET27} (Fig. S3). These results suggested that SCO3129 regulated the transcription of *SCO3128-3130* directly by interaction with the promoter region of *SCO3128*.

4. Discussion

In the natural environment, *Streptomyces* are exposed to various stress. Osmoregulation is important for their survival in the osmotic environment. The osmotic stress-induced regulation is also related with the morphological differentiation of *Streptomyces* [7,22]. The link between osmotic stress response and differentiation in *Streptomyces* has been reported in several cases, such as *catB*, *rsbA*, σ^B, σ^H and A factor [22–26]. Our results further improved the relationship of bacterial morphological differentiation and osmoregulation.

Bacteria have developed sophisticated mechanisms of stimulus perception and signal transduction in response to a variety of stress. Upon salt stress, bacterial cells gain osmotic signals and then uptake or *de novo* synthesis various compatible solutes (including glycine betaine, trehalose and ectoine) that are designed to help the cellular cytoplasm to maintain an equivalent osmotic pressure with the external environment. Both processes are part of the immediate response to osmotic stress and regulate the transcription level of osmoprotectant transporting or biosynthetic genes [27]. Although we found KCl induced the transcription of *SCO3128-3130*, we do not know whether KCl could be used as the ligand of SCO3129 in the osmoregulation. Therefore, we could not give a model

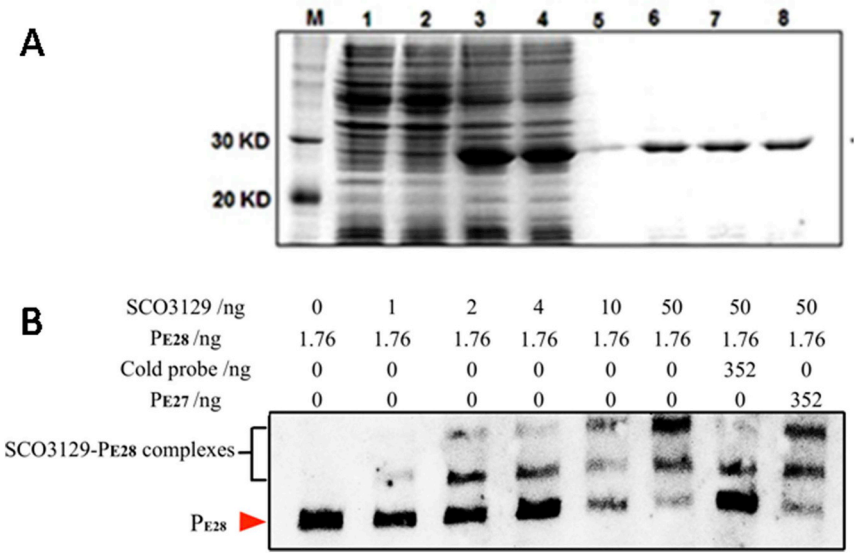

Fig. 7. Purification of His_6-SCO3129 and analysis of its binding to the promoter region of *SCO3128-3130*. A, Expression and purification of SCO3129-His_6. M, molecular size markers; 1, cellular lysate of *E. coli* BL21(DE3) carrying pET28a vector as negative control; 2, cellular lysate of *E. coli* BL21(DE3) carrying pET28a::*SCO3129*, but not induced by IPTG; 3, whole cellular proteins of *E. coli* BL21(DE3) with pET28a::*SCO3129* induced by IPTG; 4, soluble proteins of *E. coli* BL21(DE3) with pET28a::*SCO3129* induced by IPTG; 5–8, purified His_6-SCO3129 fractions from Ni-NTA affinity chromatography. B, EMSA analysis of SCO3129-His_6 binding ability to the promoter region of *SCO3128-3130*. The probe PE28 containing the upstream regions of *SCO3128* were incubated with the increasing amounts of SCO3129-His_6 (lanes 1–6 contain 0, 1, 2, 4, 10 and 50 ng proteins). EMSAs of 50 ng SCO3129-His_6 with 200 fold excess of unlabelled specific probe (cold probe) are shown in lane 7 and 200 fold excess of non-specific competitor PET27 is shown in lane 8. The arrows indicate the free probes and the braces show SCO3129 -DNA complexes.

of osmoregulation induced by KCl in *S. coelicolor* currently.

Betaine is a very common osmoprotectant that is used by many bacteria, and L-carnitine could be degraded to produce betaine which serves as osmoprotectant [28,29]. *SCO3130* encodes a putative carnitine dehydratase which has 29% identity with CaiB of *E. coli*. CaiB is the first enzyme for catalyzing the conversion of L-carnitine to γ-butyrobetaine in *E. coli* [13]. Disruption of *SCO3130* led to the mycelia lysis under the osmotic stress, indicating SCO3130 plays the same role in *S. coelicolor* as CaiB. The further study is needed to clarify the function of SCO3130, such as whether SCO3130 catalyzes the conversion of L-carnitine to γ-butyrobetaine in *S. coelicolor*.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.synbio.2018.10.012.

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