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 promoter 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.
Table 1

| Strains or plasmids | relevant characteristics | Source or reference |
|---------------------|--------------------------|---------------------|
| 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- repl F' d Vac2 ΔM15 | Gibco BRL.          |
| BL21(DE3)          | F- ompT hsd s gal d cm (DE3) | Novagen             |
| ETI2567 (pZU8002)  | recA dam dcm hsd Can' Str' Tetr' Km' | [30]              |

Plasmids

| Plasmids | Description |
|----------|-------------|
| 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 |
| pU119::neo | Provide the kanamycin resistance cassette | [9] |
| pIJ9295 | Routine cloning and subcloning vector | [8] |
| pKC1132::SCO3128::neo | SCO3128 disruption vector for neo insertion | This work |
| pKC1132::SCO3130::neo | SCO3130 disruption vector for neo insertion | This work |
| pKC1132::SCO3129::neo | SCO3129 disruption vector for neo insertion | This work |
| pKC1132::SCO3129LR | SCO3129 marker-free deletion vector to replace neo with SCO3129LR | This work |

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 ETI2567 (dam dcm hsd) was used to propagate non-methylated DNA. E. coli ETI12567 (pZU8002) was used for conjugal transfer of DNA from E. coli to Streptomyces.

2.2. Plasmids construction and DNA transformation

The plasmid pIJ9295 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 μg/mL respectively.

2.3. Construction of the SCO3128 disruption mutant

To construct the SCO3128 disruption mutant, a 2236 bp DNA fragment containing the entire SCO3128 and its flanking sequence was amplified with the PCR primers p3128DF/p3128DR. It was then digested with HindIII-sall and the DNA fragment containing SCO3128 was ligated into the corresponding sites of pIJ9295 to generate p28DM-1. The neo gene was amplified with the primers KanF/KanR using pUC119::neo as the template and was inserted into the Smal site of SCO3128 in p28DM-1. The resulting plasmid was digested with HindIII-EcoRI 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 ETI12567 (pZU8002). The kanamycin-resistance and apramycin-sensitive exconjugants were selected and the SCO3128 disruption mutant was further confirmed by PCR using the primers p3128DF/p3128DR.

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 p3129DF/p3129DR. It was then ligated into the HindII site of pIJ9295 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 HindII

Table 2

| Primers used for amplifying the neo gene | Sequences (5’→3’) |
|----------------------------------------|------------------|
| KanF                                  | ACGGTTGACTCCCTTTGATACCGTTCG |
| KanR                                  | TGAACGTGAAGACCCAGCTGTCGGC |
| Primers used for gene disruption       |                  |
| p3128DF                               | CCCAAAGCTTGACGTGAAATGGTAGGAAAG |
| p3128DR                               | ACGGTTGACGTTCGGAATACCGTTCG |
| Primers used for constructing the completement strains |                  |
| p3129DF                               | TGGTGTGTTTCACATGCCCCCTGTC |
| p3129DR                               | TGGAGGCCTGATCGACGACCTTGCC |
| p3129DF1                              | CGAGAGCTGATCGACGACCTTGCC |
| p3129DR1                              | CGGATGGATCGACGACCTTGCC |
| Primers used for confirming the read through of SCO3128-3130 |                  |
| RF                                   | TCCGACGACGTCGACGACGACGACG |
| RT                                   | CGGATGCTGACGACGACGACGACGACG |
| Primers used for constructing the SCO3128::neo |                  |
| p3129F                                | CGTCTACGATCCACGCCGCCGAGGACTC |
| p3129DR                               | TGAAGATCCACGCCGCCGAGGACCTTC |
| p3129DR1                              | CGGATGCTGACGACGACGACGACGACG |
| Primers used for gene disruption       |                  |
| p3129FR                               | CCAGATGCAGGTACGACGACGACGACG |
| p3129DR1                              | CGGATGCTGACGACGACGACGACGACG |
| Primers used for real-time RT-PCR     |                  |
| p3129FR                               | GCCGTTGTGCCTCACGTCGTCG |
| p3129DR                               | GCCGTTGTGCCTCACGTCGTCG |
| p3129DR1                              | GCCGTTGTGCCTCACGTCGTCG |
| Primers used for EMSAs                |                  |
| p3129FR                               | GCCGTTGTGCCTCACGTCGTCG |
| p3129DR                               | GCCGTTGTGCCTCACGTCGTCG |
| p3129DR1                              | GCCGTTGTGCCTCACGTCGTCG |

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site of SCO3129 in p29DM-1. The 3343 bp HindIII/EcoRI 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 apramycin-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, and then digested with HindIII/KpnI and ligated into the correspondent sites of plJ2925::SCO3129::KpnI and then ligated into the correspondent sites of pIJ2925::SCO3129::HindIII to generate the plasmid pIJ2925::SCO3129DL. The downstream region of SCO3129DL was amplified with the primers 3129DL01/3129DL02 and digested with KpnI-EcoRI, and then ligated into the correspondent sites of plJ2925::SCO3129DL to generate the plasmid pIJ2925::SCO3129DL::SCO3129DR. The 2507 bp HindIII/EcoRI fragment from plJ2925::SCO3129DL::SCO3129DR was ligated into the correspondent sites of pKC1132 to give the disruption plasmid pKC1132::SCO3129::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 apramycin-sensitive double-crossover mutant and was further confirmed by PCR with the primers 3129DF/3129DR.

2.5. Construction of the SCO3130 disruption mutant

A 3223 bp DNA fragment containing the entire SCO3130 and its flanking sequence was amplified with primers 3130DF/3130DR. Then the DNA fragment was driven by HindIII and EcoRI and ligated into the correspondent site of pJ2925 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 ApaI site of pSCO3130 in p30DM-1. The 4219 bp HindIII/EcoRI 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 apramycin-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 EcoRV 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 His6-SCO3129

The entire SCO3129 was amplified with the primers SCO3129F/SCO3129R. After digested with Ndel-Xhol, the amplified fragment was inserted into the corresponding sites of PT28a 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 OD600 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 His6-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_{KET27} containing a 150 bp upstream region of SCO3127 was amplified with the primers 3127EF/3127ER. Similarly, the probe P_{KET28} 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 His6-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 MgCl2. 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-HCl (pH 7.9), 20 mM sodium acetate and 1 mM EDTA. Then the gel was stained with 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_{KET27} was amplified by the primers RT3127F/RT3127R; the probe P_{KET28} 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 dehydrogenase) 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...
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* 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.

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*) did not show any obvious difference comparing with the wild-type M145. The *SCO3130* disruption mutant (*SCO3130DM*)...
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/MgCl2) than M145 (Fig. 3A and Fig. S2). In the MMM or MMG supplemented KCl, SCO3130DM only formed very small colonies, and its mycelial 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], TprR [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

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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 × 10⁷ 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.

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.
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 His6-tagged SCO3129 was purified to near homogeneity by a single chromatography on Ni-NTA resin (Fig. 7A). The DNA fragment PET28 covering the promoter region of SCO3128 and the DNA fragment PET27 covering the promoter region of SCO3127 were used as probes. EMSAs showed that the purified His6-tagged SCO3129 could specifically bind to the promoter region of SCO3128 (Fig. 7B), but not to PET27 (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, σH, σF 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.
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 of osmoregulation induced by KCl in *E. coli* currently. L-carnitine is the first enzyme for catalyzing the conversion of l-carnitine to γ-butyrobetaine in *E. coli* [13]. Disruption ofSCO3130 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.syntbio.2018.10.012.

References

[1] Demain AL. Pharmaceutically active secondary metabolites of microorganisms. Appl Microbiol Biotechnol 1999;52:455–63.
[2] Chater KF. Multilevel regulation of Streptomyces differentiation. Trends Genet 1998;5:372–7.
[3] Chater KF. Regulation of sporulation in *Streptomyces coelicolor* A3(2): a checkpoint multiplex?Curr Opin Microbiol 2001;4:667–73.
[4] Hesketh A, Sun J, Bibb M. Induction of ppGpp synthesis in *E. coli*. J Bacteriol 1999;181:204–11.
[5] Lee EJ, Karoonuthaisiri N, Kim HS, Park JH, Cha CJ, Kao CM, Roe JH. A master regulator sigmaB governs osmotic and oxidative response as well as differentiation via a network of sigma factors in *Streptomyces coelicolor*. Mol Microbiol 2005;57:1252–64.
[6] Takano H, Hosokos K, Beppu T, Ueda K. Involvement of sigmaH and related sigma factors in glucose-dependent initiation of morphological and physiological development of *Streptomyces griseus*. Gene 2003;320:127–35.
[7] Kiser T, Bibb MJ, Buttrner MJ, Chater KF, Hopwood DA. Practical *Streptomyces* genetics. Norwich, UK: John Innes Foundation; 2000.
[8] Zhuo J, Ma B, Xu J, Hu W, Zhang J, Tan H, Tian Y. Reconstruction of a hybrid nucleoside antibiotic gene cluster based on scarce modification of large DNA fragments. Sci China Life Sci 2017;60:968–79.
[9] Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory Press; 1989.
[10] Guan F, Pan Y, Li J, Liu G. A GATA-type transcription factor AcARRE for nitrogen metabolism is involved in regulation of cephalosporin biosynthesis in *Acinetobacter*. Sci China Life Sci 2017;60:958–67.
[11] Weng SL, Fan KQ, Yang X, Lin ZX, Xu XP, Yang KQ. CabC, an EF-hand calcium-binding protein, is involved in Ca2+-mediated regulation of spore germination and aerial hypha formation in *Streptomyces coelicolor*. J Bacteriol 2008;190:4601–8.
[12] Jung H, Jung K, Kleber HP. L-carnitine metabolism and osmotic stress response in *E. coli*. J Basic Microbiol 1992;32:409–13.
[13] Li M, Gu R, Su CC, Routth MD, Harris KC, Jewell ES, McDermott G, Yu EW. Crystal structure of the transcriptional regulator AcrR from *Escherichia coli*. J Mol Biol 2007;374:591–603.
[14] Grkovic S, Brown MH, Schumacher MA, Brennan RG, Skurray RA. The staphylococcal QacR multidrug regulator binds a correctly spaced operator as a pair of dimers. J Bacteriol 2001;183:7102–9.
[15] Lee EJ, Ueda K, Horinouchi S. A master regulator AcrR from *E. coli* interacts with the promoter region of *acrAB* and *mtrR* genes of *Neisseria gonorrhoeae*. J Bacteriol 2001;183:7330–3.
[16] Lucas CE, Balbazar JT, Hagman KE, Shafer WM. The MrIR repressor binds the DNA sequence between the marR and merC genes of *Neisseria gonorrhoeae*. J Bacteriol 1997;179:4123–8.
[17] Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory Press; 1989.
[18] Lucas CE, Balbazar JT, Hagman KE, Shafer WM. The MrIR repressor binds the DNA sequence between the marR and merC genes of *Neisseria gonorrhoeae*. J Bacteriol 1997;179:4123–8.
[19] Ando N, Horinouchi S. A Strepotmyces griseus gene (sga) suppresses the growth disturbance caused by high osmolality and a high concentration of A-factor. Microbiology 1997;143:2715–23.
[20] Cho YH, Lee EJ, Roe JH. A developmentally regulated catalase required for proper differentiation and osmoprotection of *Streptomyces coelicolor*. Mol Microbiol 2000;35:510–60.
[21] Su CC, Rutherford DJ, Yu EW. Characterization of the multidrug efflux regulator AcrR from *Escherichia coli*. Biochim Biophys Acta 2007;1761:85–90.
[22] Rebouche CJ, Seim H. Carnitine metabolism and its regulation in microorganisms and mammals. Annu Rev Nutr 1998;18:39–61.
[23] Paget MS, Chamberlin L, Atrih A, Foster SJ, Buttnner MJ. Evidence that the extra-cytoplasmic function sigma factor sigmaE is required for normal cell wall structure in *Streptomyces coelicolor* A3(2). J Bacteriol 1999;181:204–11.