DptR2, a DeoR-type auto-regulator, is required for daptomycin production in *Streptomyces roseosporus*

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Daptomycin production  
Transcriptional regulator  
Auto-regulation

**Abstract**  
Daptomycin, a novel cyclic lipopeptide antibiotic against Gram-positive bacteria, is produced by *Streptomyces roseosporus*. Though its biosynthetic mechanism, structural shuffling and fermentation optimization have been extensively studied, little is understood about its production regulation at the transcriptional levels. Here we reported that dptR2, encoding a DeoR-type regulator located close to the daptomycin biosynthesis gene cluster in *S. roseosporus* SW0702, is required for daptomycin production, but not for the expression of daptomycin gene cluster, suggesting that DptR2 was not a pathway-specific regulator. Furthermore, EMSA and qRT-PCR analysis suggested that DptR2 was positively auto-regulated by binding to its own promoter. Meanwhile, the binding sites on the dptR2 promoter were determined by a DNase 1 footprinting assay, and the essentiality of the inverted complementary sequences in the protected region for DptR2 binding was assessed. Our results for the first time reported the regulation of daptomycin production at the transcriptional level in *S. roseosporus*.  
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**1. Introduction**  
Daptomycin is a cyclic lipopeptide antibiotic against a broad spectrum of Gram-positive bacteria. It is the main active component of Cubicin, approved by FDA for treatment of complicated skin infections in 2003 (Kirkpatrick et al., 2003) and *Staphylococcus aureus* infections of bacteremia and endocarditis in 2006 (Fowler et al., 2006; Nguyen et al., 2006b). Daptomycin is produced by *S. roseosporus* in an A2197BC complex via a non-ribosomal peptide synthetase (NRPS) pathway. A2197BC contains a universal structure of a 10 amino acids ring formed by an ester bond between the carboxyl group of L-kynurenine 13 (l-kyn13) and the hydroxyl group of L-thr4, and a three amino acid tail with fatty acids attached to the terminal amino group of L-trp1 (Miao et al., 2005). Daptomycin is the only product with the straight-chain decanoic acid, and in industry, this fatty acid is added to the fermentation culture of *S. roseosporus* to improve daptomycin production (Huber et al., 1988).

In *S. roseosporus* NRRL 11379, the gene cluster for daptomycin biosynthesis has been cloned and the functions of several structure genes have been experimentally elucidated (Liao et al., 2013; Miao et al., 2005; Nguyen et al., 2006a; Wittmann et al., 2008). Meanwhile, chemical, chemo-enzymatic and combinational biosynthetic methods have been developed to generate a variety of novel daptomycin derivatives to screen more potentially potent antibiotics (Grunewald et al., 2004; Nguyen et al., 2006b). Moreover, many efforts have been also made to increase daptomycin production, including ribosome engineering (Li et al., 2013), random mutagenesis (Yu et al., 2011) and introduction of extra copies of some dpt genes, such as dptJ (Liao et al., 2013), etc. However, the molecular regulatory mechanism of daptomycin production at the transcriptional levels has not been investigated. Our results sequencing has revealed three possible regulatory genes, dptR1, dptR2 and dptR3, which are located very close to the dpt gene cluster, but their functions have not been reported (Liao et al., 2012; Miao et al., 2005).

DeoR-type transcriptional regulators are named after *E. coli* deo operon (Mortensen et al., 1989). Most DeoR-type regulators function as repressors, such as UlaR and AgaR for transcriptional repression of l-ascorbate and galactosamina metabolism in *E. coli*, respectively (Garces et al., 2008; Ray and Larson, 2004). G1pR, responsible for glycerol catabolism repression in *E. coli* and *Rhizobium leguminosarum*, respectively (Deng et al., 2012; Schweizer et al., 1985), and LacR, a repressor of the lactose phosphotransferase
system of Lactococcus lactis (van Rooijen and de Vos, 1990). However, FurR acts as an activator of fructose operon transcription in Spiroplasma citri (Gaurivaud et al., 2001). Recently, it has been suggested that DeoR-citri FruR acts as an activator of fructose operon transcription in D. type regulators were prevalent in S. roseosporus avermitilis, which has been shown to require daptomycin production in an industrial strain (Ulanova et al., 2013). Here we reported that DptR2, a DeoR-family regulator, is required for daptomycin production in an industrial strain S. roseosporus SW0702, for the first time providing evidence at the transcriptional level for daptomycin production.

2. Materials and methods

2.1. Strains and media

Strains used in this study are listed in Table 1. E. coli strains were cultured in LB medium at 37 °C. S. roseosporus strains were grown at 30 °C on solid R5 medium (Kieser et al., 2000) for sporulation. For daptomycin production, liquid 2% TSB plus 5% PEG6000 was used as the seed medium and the liquid YEME medium (0.3% yeast extract, 0.3% malt extract, 0.5% tryptone, 4% glucose) was used as the fermentation medium. The feeding medium contained 50% decanoic acid and 50% methyl oleate.

2.2. Plasmid construction

Plasmids and primers used in this work are described in Tables 2 and 3, respectively. dptR2DBD was amplified with primer pair 1, 2, ligated into pTA2 after dA addition with EcoRI and digested with EcoRI, to generate plasmids dptR2p and pIJ8660-pECI from pTA2. The dptR2p fragment was then ligated into the BamHI/Xhol site of pET28a for pET28a-dptR2DBD. Two 1 kb DNA fragments spanning the dptR2 coding region were amplified from the genomic DNA of S. roseosporus SW0702, with primer pair 3 and primer pair 5, 6, respectively. The resulting DNA fragments were ligated into pTA2 after dA addition, digested with HindIII/BamHI and BglII/EcoRI, respectively, and sequentially ligated into pKC1139 (Bierman et al., 1992), generating the disruption plasmid pKC1139-dΔptR2. To construct the complementation plasmid pSET152-dptR2p + dptR2, primers 7 and 9 were used to amplify the dptR2 coding region with its promoter. The PCR product was inserted into pTA2, digested with BglII/EcoRI and ligated to BamHI/EcoRI site of pSET152 (Bierman et al., 1992). The dptR2p and dptR1p were amplified with primer pair 7, 8, and primer pair 10, 11, and ligated to pTA2 to generate plasmids pTA2-dptR2p and pTA2-dptR1p, respectively. The dptR2p from pTA2-dptR2p digested with BglII was inserted into BamHI site of pUC18 and BglII site of pJ8660 (Sun et al., 1999) to construct plasmids pUC18-dptR2p and pJ8660-dptR2p, respectively. The dptR1p from pTA2-dptR1p digested with BglII was inserted into BamHI site of pUC18 to generate plasmid pUC18-dptR1p. Oligonucleotides listed in Table 3, 11, and 14 were annealed, respectively, by heating at 10 mM each to be used as oligonucleotides.

Table 1

<table>
<thead>
<tr>
<th>Strains</th>
<th>Description</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Streptomyces roseosporus SW0702</td>
<td>Wild type daptomycin producer</td>
<td>This study</td>
</tr>
<tr>
<td>ΔdptR2</td>
<td>dptR2 disruption mutant</td>
<td>This study</td>
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<tr>
<td>E. coli TGI</td>
<td>A general cloning host</td>
<td>Novagen</td>
</tr>
<tr>
<td>E. coli ET12567/pUZ8002</td>
<td>Methylation-deficient E. coli for conjugation with the helper plasmid</td>
<td>Novagen</td>
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<tr>
<td>E. coli BL21 (DE3)</td>
<td>A host for protein expression</td>
<td>Novagen</td>
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</table>

Table 2

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<tr>
<th>Plasmids</th>
<th>Description</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>pTA2</td>
<td>T vector</td>
<td>Toyobo, Japan</td>
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<tr>
<td>pTA2-dptR2p</td>
<td>pTA2 containing the promoter of dptR2</td>
<td>This study</td>
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<tr>
<td>pUC18</td>
<td>Vector</td>
<td>Invitrogen</td>
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<tr>
<td>pUC18-dptR1p</td>
<td>pUC18 containing the promoter of dptR1</td>
<td>This study</td>
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<tr>
<td>pUC18-dptR2p</td>
<td>pUC18 containing the promoter of dptR2</td>
<td>This study</td>
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<td>pUC18-dptR3pL</td>
<td>pUC18 containing fragment dptR2pL</td>
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<td>pUC18 containing fragment dptR2pM4</td>
<td>This study</td>
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<td>pKC1139</td>
<td>Temperature-sensitive shuttle vector for gene disruption in Streptomyces</td>
<td>Bierman et al. (1992)</td>
</tr>
<tr>
<td>pKC1139-dΔptR2</td>
<td>Expression vector in E. coli</td>
<td>Novagen</td>
</tr>
<tr>
<td>pET28a-dptR2DBD</td>
<td>pET28a containing the coding region of dptR2 DNA binding domain</td>
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<tr>
<td>pSET152</td>
<td>Integrative shuttle vector</td>
<td>Bierman et al. (1992)</td>
</tr>
<tr>
<td>pSET152 + dptR2p-dptR2</td>
<td>dptR2 complementation plasmid based on pSET152</td>
<td>This study</td>
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<tr>
<td>pJ8660</td>
<td>Promoter-probing plasmid</td>
<td>Sun et al. (1999)</td>
</tr>
<tr>
<td>pJ8660-dptR2p</td>
<td>pJ8660 containing 291 bp of dptR2</td>
<td>This study</td>
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</table>
95 °C for 10 min on a thermocycler and cooling down to room temperature slowly. Then, the fragments were ligated into BamHI/EcoRI site of pUC18 for plasmids pUC18-dptR2pL, pUC18-dptR2pLM1, pUC18-dptR2pLM2, pUC18-dptR2pLM3 and pUC18-dptR2pLM4, respectively.

2.3. Protein expression and purification

E. coli BL21 (DE3) cells containing plasmid pET28a-dptR2DBD were grown at 37 °C to OD600 = 0.6, and then induced by 0.1 mM IPTG at 16 °C overnight. The resulting His-tagged DptR2DBD was purified by nickel affinity chromatography according to the manufacturer's instruction (Qiagen). The purified fusion protein was assessed by SDS-PAGE and the concentration was determined by the Bradford method (Kruger, 1994).

2.4. Construction of S. roseosporus strains

S. roseosporus strains used in this study are listed in Table 1. In order to inactivate dptR2, the plasmid pKC1139-ΔdptR2 was introduced into S. roseosporus SW0702 via E. coli ET12567/pUZ8002 (Macneil and Klapko, 1987). The resulting transformants were streaked on R5 medium to obtain the complementation strain. Southern blotting analysis was used to confirm the genotype of ΔdptR2 disruptant. For complementation of dptR2, plasmid pSET152-dptR2p + dptR2 was introduced into ΔdptR2 via E. coli ET12567/pUZ8002 (Macneil and Klapko, 1987) to obtain the complementation strain ΔdptR2 + dptR2.

2.5. Southern blot

To verify the deletion of dptR2, Southern blot was performed as before with minor modifications (Cariani and Brechot, 1988). Briefly, 10 μg of genomic DNA was separated in a 0.8% agarose gel after digested with PvuII and BamHI, respectively. The DNA in gel was denatured by being incubated in 0.5 M NaOH and 1.5 M NaCl for 30 min, washed with distilled water, and neutralized with 1.5 M NaCl and 0.5 M Tris-HCl, pH 7.2, for 30 min. Subsequently, the DNA fragments were capillarily transferred to nylon membranes in 10 × SSC (87.65 g/L NaCl, 44.1 g/L sodium citrate, pH 7.0), and fixed by UV. The membrane was prehybridized for 1 h at 42 °C in prehybridization solution (50% formamide, 5 × SSC, 5 × Denhardt’s solution, 50 mM sodium phosphate, pH 6.5, 100 μg/mL denatured sonicated salmon sperm DNA) and hybridized with the probe prepared by PCR with primers 3 and 4 in the presence of biotin-11-dUTP (Fermentas) from template pKC1139-ΔdptR2 in prehybridization solution at 42 °C overnight. After washed three times with 2 × SSC and 0.1% SDS at 42 °C, the probe was detected with streptavidin-AP and CDP-STAR (Roche).

2.6. Electrophoretic mobility shift assay (EMSA)

The EMSA was performed as described before (Mao et al., 2013). The 5′-biotin-labeled dptR2p, dptR1p, dptR2pL, dptR2pLM1, dptR2pLM2, dptR2pLM3, and dptR2pLM4 probes were obtained by PCR using primer

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**Fig. 1.** Gene organization in a 56 kb region containing the dpt gene cluster and its adjacent genes from dptM to orf53. The thick bottom lines represent the promoter regions used in this study.

**Fig. 2.** Confirmation of dptR2 disruption. (A) Predicted PvuII or BamHI restriction sites on dptR2 locus. The probe for Southern blot is indicated. (B) Southern blot analysis of wild type and ΔdptR2 mutant strains. Genomic DNA was digested with PvuII or BamHI, respectively. Wild type strain showed a hybridized band of 5.4 kb, and ΔdptR2 showed a 4.3 kb band when Genomic DNA was digested with BamHI and Wild type strain showed a hybridized band of 3.2 kb, and ΔdptR2 showed a 5.2 kb band when Genomic DNA was digested with PvuII.

**Fig. 3.** DptR2 positively regulates daptomycin biosynthesis. (A) Daptomycin production analysis of wild type, ΔdptR2 and complementation strain by HPLC. (B) DptR2 did not regulate gene expression of dptA to hrdB as measured by qRT-PCR in two independent experiments.
pair 22, 23 from plasmids pUC18-dptR2p, pUC18-dptR1p, pUC18-dptR2pL, pUC18-dptR2pLM1, pUC18-dptR2pLM2, pUC18-dptR2pLM3, and pUC18-dptR2pLM4, respectively. Binding of DptR2DBD to DNA probes was performed in 10 μL of reactions containing 1 ng of DNA probe and different amounts of DptR2DBD. After 30 min of incubation at 30 °C, the reaction mixtures were applied to a 5% native polyacrylamide gel with 0.5 × TBE as running buffer at 80 V for 1–3 h. Subsequently, DNA probes were electro-blotted onto a nylon membrane in 0.5 × TBE buffer at 250 mA for 1 h, fixed by UV and detected with streptavidin-HRP and BeyoECL plus (Beyotime, China).

2.7. DNase I footprinting assay

DNase I footprinting assay was performed by fluorescent labeling procedure as described before (Mao et al., 2013; Santos-Aberturas et al., 2011). Briefly, purified DptR2DBD protein was ultra-filtered with YM-3 (Millipore) for the 3 kD cut-off, washed twice and eluted with 0.5 M NH₄Ac, 40 mM Tris, pH 8.0. After extraction with phenol/chloroform, and precipitation at 214 nm (SPD-20A; Shimadzu), the mobile phase consisted of 65% solution A (0.05 M Na₂HPO₄ (pH 3.15 ± 0.05)) and solution B (100% acetonitrile) with UV detection at 214 nm (SPD-20A; Shimadzu). The DNA sequencing ladder was prepared with universal primer 24 according to Thermo Sequence Dye Primer Manual Cycle Sequencing Kit (USB).

2.8. HPLC analysis of daptomycin

Spores of S. roseosporus SW0702 and its derivatives prepared from R5 medium were inoculated into TSB. The cultures were grown at 30 °C on a rotary shaker at 250 rpm for about 30 h as the seed culture. 1 mL of seed culture was inoculated into flasks containing 35 mL of YEME medium and then fermented at 30 °C on a rotary shaker at 250 rpm for 5 days. From 36 h to 5 days fermentation, the feeding medium was added to the fermentation medium (1:1000, v/v) twice per day. The culture was harvested by centrifugation and the supernatant was filtered through a Millipore membrane. Daptomycin was analyzed by HPLC with a C₁₈ reverse phase column (Zorbax 300SB-C₁₈, 5 μm, 4.6 by 250 mm; Agilent) with solution A (0.05 M Na₂HPO₄ (pH 3.15 ± 0.05)) and solution B (100% acetonitrile) with UV detection at 214 nm (SPD-20A; Shimadzu). The mobile phase consisted of 65% solution A and 35% solution B and the flow rate was 1.0 mL/min. Pure daptomycin was used as a standard.

2.9. RNA analysis

Total RNA was extracted by Trizol according to the manufacture’s instruction (Sangon) from mycelia of S. roseosporus SW0702 and its derivative strains, which were collected in YEME medium for the time indicated (48, 72, 96 and 120 h), and treated with RNase-free DNase I (TaKaRa) to remove genomic DNA. cDNA was synthesized with MMLV reverse transcriptase as described by the manufacturer’s protocol (TaKaRa). Quantitative real-time PCR (qRT-PCR) was performed with SYBR PremixEx Taq II (TaKaRa). Primer pair 25, 26, primer pair 27, 28 were used to detect the expression abundance of dptA, egfp, and hrdB, respectively. The sigma factor gene hrdB was used as an internal control. Fold changes of egfp and dpta expression were calculated using the comparative Ct method with the formula $2^{-ΔΔCt}$ according to the protocol (TaKaRa).

2.9.1. Accession numbers

The dptR2 gene and its promoter region, dptR1 gene promoter region, dpta gene and hrdB gene sequences were deposited in GenBank under accession numbers KJ126785, KJ126786, KJ126787 and KJ126788, respectively.
3. Results and discussion

3.1. DptR2 positively regulates daptomycin biosynthesis

It has been reported that \textit{S. roseosporus} NRRL 11379 contains the daptomycin gene cluster and can produce the A21978C complex including daptomycin (Miao et al., 2005). In this report, whole genome sequencing revealed that \textit{S. roseosporus} SW0702 strain, an industrial daptomycin producer, also contained the daptomycin gene cluster. All the genes were highly conserved with those in \textit{S. roseosporus} NRRL 11379, and the gene arrangement was identical (Fig. 1).

\textit{dptR2}, located close to the \textit{dpt} gene cluster, encodes a protein with 371 amino acids. BLAST showed that DptR2 had a DeoR family helix–turn–helix (HTH) DNA binding domain at its N-terminus, and the C-terminus belonged to the type 1 periplasmic-binding fold protein superfamily, a ligand binding domain of the LacI transcriptional regulator family (PBP1_LacI_substrate binding domain), which also acts as an oligomerization domain. Considering its adjacency to the \textit{dpt} gene cluster, it was supposed as one of the pathway-specific regulators for daptomycin production (Miao et al., 2005).

\textit{dptR2} was disrupted in \textit{S. roseosporus} SW0702 by in-frame deletion strategy (Fig. 2A), and the genotypes were confirmed by Southern blot (Fig. 2B). In YEME as the fermentation medium, \textit{ΔdptR2} mutant did not produce daptomycin, and complementation with \textit{dptR2} under its native promoter could restore the production of daptomycin to a similar level to wild type (Fig. 3A). These results suggested that DptR2 played a positive role on daptomycin production in \textit{S. roseosporus} SW0702.

Most pathway-specific regulators can directly bind to the gene cluster to regulate gene expression (Gomez et al., 2012). Genes from \textit{dptE} to \textit{dptH} are transcribed as poly-cistronic units as reported previously, and

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure6.png}
\caption{DNase I footprinting assay for DptR2DBD binding site determination. (A) 5’-FAM-labeled \textit{dptR2p} probe was used in the DNase I footprinting assay without or with purified DptR2DBD. Two protected sites (\textit{dptR2pL} and \textit{dptR2pR}) were determined with 4 μg of DptR2DBD. (B) Sequence of the protected \textit{dptR2} promoter regions. The reactions without and with DptR2DBD are indicated in blue and red, respectively. The sequencing reaction is shown at the bottom and the corresponding protected regions are underlined. (C) The promoter regions of \textit{dptR2}. The DptR2 binding sites deduced from the DNase I footprinting assay are underlined and italic, and the translation start codon is boxed. The inverted complementary repeats are shown in bold.}
\end{figure}
**dptM** and **dptN**, encoding ABC transporters (Miao et al., 2005), **dptI** and **dptJ**, participating in formation of L-3-methylglutamic acid and Kyn (Liao et al., 2013; Nguyen et al., 2006a), might be expressed as a bicistronics, respectively. However, we did not find significant difference of the gene expression profile of **dpt** genes, represented by **dptA**, encoding one subunit of daptomycin NRPS (Miao et al., 2005), between wild type and **ΔdptR2** mutant cultured in YEME medium for 2–5 days, respectively (Fig. 3B). These results suggested that the lack of daptomycin production in **ΔdptR2** mutant did not result from the transcriptional deficiency of the **dpt** genes. Meanwhile, our EMSA showed that DptR2 could bind to neither the inter-genic regions before **dptM**, **dptE** or **dptI** (data not shown), nor the promoter region of another putative pathway-specific regulator gene **dptR1** (Figs. 1 and 5B), suggesting that DptR2 might not function as a **dpt** gene cluster pathway-specific regulator.

DptR2 has a PBP1_LacI_substrate binding domain for monosaccharide binding. This led to the possibility that **dptR2** disruption may affect monosaccharide metabolism, such as glucose, resulting in the lack of precursors or energy supply for daptomycin biosynthesis. The complete disappearance of daptomycin in **ΔdptR2** mutant suggested that DptR2 might be a vital regulator for daptomycin production, so it might be of interest to unravel the DptR2 regulons by chromatin immunoprecipitation (ChIP), etc. (Bush et al., 2013).

### 3.2. **DptR2 is a positive auto-regulator**

Since DptR2 played a positive role on daptomycin production, we next checked the expression profile of **dptR2** during secondary metabolism with a promoter-probing reporter system by transcriptional fusion of the promoter region of **dptR2** (**dptR2p**) to **egfp** (Sun et al., 1999). The expression levels of egfp in both wild type and **ΔdptR2** mutant were assayed by qRT-PCR from 2 to 5 days in YEME medium for daptomycin production. It was observed that there was slight but significant decrease of **egfp** expression levels in **ΔdptR2** mutant at 72, 96 and 120 h, respectively (Fig. 4), suggesting that DptR2 was positively auto-regulated.

### 3.3. **DptR2 binds to its own promoter region**

We further determined whether DptR2 regulated its own expression through binding to its promoter region. The DNA-binding domain of DptR2 (DptR2DBD) was overexpressed in *E. coli* BL21 (DE3) and purified as a His-tagged protein (Fig. 5A). EMSA showed that purified DptR2DBD could bind to a 289 bp fragment encompassing the intergenic region between **dptR2** and **orf53** in a dosage dependent manner, but not to the **dptR1** promoter as a control (Fig. 5B), suggesting a specific interaction between DptR2DBD and its own promoter. Our results indicated that DptR2 positively regulated its expression by binding to its own promoter.

DNase I footprinting assay was demonstrated to determine the binding regions of DptR2DBD on **dptR2** promoter. Two binding sites were protected by DptR2DBD on its promoter region, **dptR2p**

\[\text{WT: GCGAAATGAGATCGATCA} \quad \text{GGATCGATAGGAAAGCAGCTAGTCAACGAGCTCC} \]

\[\text{M1: GCGAAATGAGATCGATCA} \quad \text{GGATCGATAGGAAAGCAGCTAGTCAACGAGCTCC} \]

\[\text{M2: GCGAAATGAGATCGATCA} \quad \text{GGCTCAAGGAAAGCAGCTAGTCAACGAGCTCC} \]

\[\text{M3: GCGAAATGAGATCGATCA} \quad \text{TTGGATCGATAGGAAAGCAGCTAGTCAACGAGCTCC} \]

\[\text{M4: GCGAAATGAGATCGATCA} \quad \text{CGATCGATAGGAAAGCAGCTAGTCAACGAGCTCC} \]

(Fig. 5A,B) extending from −183 to −223 and −10 to −46 relative to the translation start codon of **dptR2**, respectively. A palindromic sequence, formed by the inverted complementary sequences (ATCGATC and GATCGAT) that were separated by two nucleotides (AG), was found in **dptR2p**

\[\text{WT: GCGAAATGAGATCGATCA} \quad \text{GGATCGATAGGAAAGCAGCTAGTCAACGAGCTCC} \]

\[\text{M1: GCGAAATGAGATCGATCA} \quad \text{GGATCGATAGGAAAGCAGCTAGTCAACGAGCTCC} \]

\[\text{M2: GCGAAATGAGATCGATCA} \quad \text{GGCTCAAGGAAAGCAGCTAGTCAACGAGCTCC} \]

\[\text{M3: GCGAAATGAGATCGATCA} \quad \text{TTGGATCGATAGGAAAGCAGCTAGTCAACGAGCTCC} \]

\[\text{M4: GCGAAATGAGATCGATCA} \quad \text{CGATCGATAGGAAAGCAGCTAGTCAACGAGCTCC} \]

(Fig. 5C). These two binding sites were separated by a large region of 136 bp (Fig. 6C). It has been reported that the DeoR-type regulators usually bind to several regions with a long spacing...
within the target promoters. DeoR repressors cooperatively bind to three sites O1, O2, separated by 599 bp and O3, 279 bp in front of the O1 within the promoter region of the deo operon, generating a double loop structure, and finally achieving full repression of the deo operon in E. coli. (Majumdar and Adhya, 1984; Mortensen et al., 1989). LacR in E. coli also has three DNA binding site: one primary site (O1) and two auxiliary sites located 92 bp upstream (O3) and 401 bp downstream (O2) relative to O1, facilitating the DNA looping (Zhang et al., 2006). Here, the 136 bp region between two DptR2DBD binding sites may also provide a flexible loop for transcriptional activation by DptR2.

3.4. Mutation analysis of DptR2DBD binding regions

Next, how the palindromic sequence affected the binding affinity of DptR2DBD for the promoter was investigated. A 50 bp fragment derived from dptR2p containing the dptR2pL sequence was chosen as the wild type probe of dptR2pL for EMSA. Four mutated probes named M1, M2, M3 and M4 were constructed (Fig. 7A). M1 altered the inverted repeat to direct repeat, while the inverted repeat sequence in M2 was randomly rearranged. Two extra base pairs were inserted in the spacer region between the inverted complementary sequences in M3. For M4, the spacer DNA was changed from “AG” to “TC”. EMSA showed that the mutations in M1 and M2 probes caused complete loss of DptR2DBD binding. Although DptR2DBD could still bind to M3 and M4 probes, it required higher concentration of DptR2DBD compared to the wild type probe (Fig. 7B). These results suggested that the palindromic sequence in the binding site was indispensable for DptR2 binding, and that the spacer sequence and length were also important for the effective interaction between DptR2 and its promoter.

Besides DptR2, many DeoR-type regulators binding sites contain palindromic sequences. For instance, in E. coli, a 16 bp palindromic sequence exists in DeoR binding sites O1 and O2 of deo operon, respectively (Mortensen et al., 1989). And GlnR binding sites also have a conserved 20 bp palindromic sequence (Larson et al., 1992). DeoR-type regulators have a C-terminal oligomerization domain and often function in the form of oligomers. For example, DeoR arranges in an octamer to exert its negative function for deo operon, and LacR can bind to two lac operator DNA fragments as a tetramer in E. coli. Besides, both GlnR and AgaR act as tetrayers in E. coli (Garces et al., 2008). So it is possible that DptR2 also form an oligomer to bind to its operators.

4. Conclusions

Here in the industrial daptomycin producer S. roseosporus SW0702, we have identified regulator gene dptR2 located close to the dpt gene cluster. Though DptR2 was required for daptomycin production, it did not regulate the expression of dpt gene cluster, suggesting that it was not a dpt gene cluster-specific regulator, which was a contrast to the previous prediction. Besides, DptR2 was positively auto-regulated by binding to its own promoter directly. Two binding sites of DptR2DBD on dptR2 promoter separated by a region of 136 bp were detected, and the palindromic sequence in the binding sites was indispensable for DptR2 binding by mutation analysis.

Conflict of interest statement

We declare that we have no conflict of interest.

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