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

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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 I 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*.

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 A21978C complex via a non-ribosomal peptide synthetase (NRPS) pathway. A21978C contains a universal structure of a 10 amino acids ring, formed by an ester bond between the carboxyl group of l-kynurenine13 (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. Gene cluster 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 DeoR, a repressor of *Escherichia 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), GlpR, responsible for glycerol catabolism repression in *E. coli* and *Rhizobium leguminosarum*, respectively (Ding et al., 2012; Schweizer et al., 1985), and *LacR*, a repressor of the lactose phosphotransferase

Abbreviations: A, adenosine; bp, base pair(s); C, cytidine; cDNA, DNA complementary to RNA; DBD, DNA binding domain; DeoR, deoxyribose regulator; DNase, deoxyribonuclease; dNTP, deoxyribonucleoside triphosphate; *egfp*, enhanced green fluorescent protein; EMSA, electrophoretic mobility shift assay; G, guanosine; HPLC, high-performance liquid chromatography; HRP, horseradish peroxidase; IPTG, isopropyl β-D-thiogalactopyranoside; kb, kilobase(s) or 1000 bp; kD, kilodalton(s); Kyn, kynurenine; LB, Luria–Bertani (medium); NRPS, non-ribosomal peptide synthetase; PAGE, PA-gel electrophoresis; PBP, periplasmic-binding fold protein; PEG, poly(ethylene glycol); RNase, ribonuclease; rpm, revolutions per minute; SDS, sodium dodecyl sulfate; T, thymidine; Thr, threonine; Trp, tryptophan; u, unit(s); UV, ultraviolet; WT, wild type.

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Table 1
Strains used in this study.

Strains	Description	Reference
Streptomyces roseosporus SW0702	Wild type daptomycin producer	This study
Δ*dptR2*	*dptR2* disruption mutant	This study
E. coli TG1	A general cloning host	Novagen
E. coli ET12567/pUZ8002	Methylation-deficient *E. coli* for conjugation with the helper plasmid	Macneil and Klapko (1987)
E. coli BL21 (DE3)	A host for protein expression	Novagen

system of *Lactococcus lactis* (van Rooijen and de Vos, 1990). However, FruR acts as an activator of fructose operon transcription in *Spiroplasma citri* (Gaurivaud et al., 2001). Recently, it has been suggested that DeoR-type regulators were prevalent in *Streptomyces*, ranging from 7 to 17 in the sequenced *Streptomyces* genomes. However, only a few of them have been studied. For example, SdrA positively regulates the morphological development and avermectin production in *Streptomyces avermitilis*, but negatively regulates oligomycin and filipin production (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 *Taq* polymerase (TaKaRa), and digested with *Bgl*II/*Xho*I. The *dptR2DBD* fragment was then ligated into the *Bam*HI/*Xho*I site of pET28a for pET28a-*dptR2DBD*. Two 1 kb DNA fragments flanking the *dptR2* coding region were amplified from the genomic DNA of *S. roseosporus* SW0702, with primer pair 3, 4 and primer pair 5, 6, respectively. The resulting DNA fragments were ligated into pTA2 after dA addition, digested with *Hin*dIII/*Bam*HI and *Bgl*II/*Eco*RI, respectively, and sequentially ligated into pKC1139 (Bierman et al., 1992), generating the disruption plasmid pKC1139-Δ*dptR2*. 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 *Bgl*II/*Eco*RI, and ligated to *Bam*HI/*Eco*RI 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 *Bgl*II was inserted into *Bam*HI site of pUC18 and *Bgl*II site of pIJ8660 (Sun et al., 1999) to construct plasmids pUC18-*dptR2p* and pIJ8660-*dptR2p*, respectively. The *dptR1p* from pTA2-*dptR1p* digested with *Bgl*II was inserted into *Bam*HI site of pUC18 to generate plasmid pUC18-*dptR1p*. Oligonucleotide pair 12, 13, pair 14, 15, pair 16, 17, pair 18, 19 and pair 20, 21 at 10 mM each were annealed, respectively, by heating at

Table 3
Oligonucleotides used in this study.

No.	Sequence(5′–3′)
1	AGATCTCACCACCACCACCACCACATGGCACTCGCATCGGAACG
2	CTCGAGGACGAACGCCTGGTCGGGC
3	TAAAAGCTTGGCGATGTCCCACCAGGGGTC
4	TGTGGATCCCCGTTCCGATGCGAGTGCCATC
5	TGGAGATCTCGGTGAAGGCGGGCTGAGGG
6	CTAGAATTCCCTGCATTGACAGGTGACAATTCC
7	AGATCTCAGGGGTTTCCTTTCGTGC
8	AGATCTCCCGGCCTCTCCTTTTCCTT
9	GAATTCTCACCGTCCCGCCGAGAC
10	AGATCTGATGAACTCCGCACAGTTG
11	AGATCTCCAGGTGCTGGGCGAGGGC
12	AATTGCGAAATGAATCGATCAGGATCGATAGGAAAGCAAAGCTCAACGAGCTCC
13	GATCGGAGCTCGTTGAGCTTTGCTTTCCTATCGATCCTGATCGATTCATTTCGC
14	AATTGCGAAATGAGATCGATAGGATCGATAGGAAAGCAAAGCTCAACGAGCTCC
15	GATCGGAGCTCGTTGAGCTTTGCTTTCCTATCGATCCTATCGATCTCATTTCGC
16	AATTGCGAAATGAGATCGATAGCCTACGAAGGAAAGCAAAGCTCAACGAGCTCC
17	GATCGGAGCTCGTTGAGCTTTGCTTTCCTTCGTAGGCTATCGATCTCATTTCGC
18	AATTGCGAAATGAATCGATCATTGGATCGATAGGAAAGCAAAGCTCAACGAGCTCC
19	GATCGGAGCTCGTTGAGCTTTGCTTTCCTATCGATCCAATGATCGATTCATTTCGC
20	AATTGCGAAATGAATCGATCTCGATCGATAGGAAAGCAAAGCTCAACGAGCTCC
21	GATCGGAGCTCGTTGAGCTTTGCTTTCCTATCGATCGAGATCGATTCATTTCGC
22	Biotin-GCCAGGGTTTTCCCAGTCACGA
23	GAGCGGATA ACAATTTCACACAGG
24	6FAM-GTTGTAAAACGACGGC
25	CTACCGTGACTCCGACAACCATC
26	CGACGAGCAGTGACGACCAG
27	GAAGAACGGCATCAAGGTG
28	GCTTCTCGTTGGGGTCTTTG
29	CCGAGTCCGAATCTGTGATG
30	GAGGATCTGGTTGAGGCTGC

Table 2
Plasmids used in this study.

Plasmids	Description	Reference
pTA2	T vector	Toyobo, Japan
pTA2-*dptR2p*	pTA2 containing the promoter of *dptR2*	This study
pUC18	Cloning vector	Invitrogen
pUC18-*dptR1p*	pUC18 containing the promoter of *dptR1*	This study
pUC18-*dptR2p*	pUC18 containing the promoter of *dptR2*	This study
pUC18-*dptR2p*L	pUC18 containing fragment *dptR2p*L	This study
pUC18-*dptR2p*LM1	pUC18 containing fragment *dptR2p*LM1	This study
pUC18-*dptR2p*LM2	pUC18 containing fragment *dptR2p*LM2	This study
pUC18-*dptR2p*LM3	pUC18 containing fragment *dptR2p*LM3	This study
pUC18-*dptR2p*LM4	pUC18 containing fragment *dptR2p*LM4	This study
pKC1139	Temperature-sensitive shuttle vector for gene disruption in *Streptomyces*	Bierman et al. (1992)
pKC1139-Δ*dptR2*	*dptR2* disruption plasmid based on pKC1139	This study
pET28a	Expression vector in *E. coli*	Novagen
pET28a-*dptR2DBD*	pET28a containing the coding region of *dptR2* DNA binding domain	This study
pSET152	Integrative shuttle vector	Bierman et al. (1992)
pSET152 + *dptR2p-dptR2*	*dptR2* complementation plasmid based on pSET152	This study
pIJ8660	Promoter-probing plasmid	Sun et al. (1999)
pIJ8660-*dptR2p*	pIJ8660 containing 291 bp of *dptR2p*	This study