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TetR family regulator AbrT controls lincomycin production and morphological development in *Streptomyces lincolnensis*

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Abstract

Background The TetR family of transcriptional regulators (TFRs), serving as crucial regulators of diverse cellular processes, undergo conformational changes induced by small-molecule ligands, which either inhibit or activate them to modulate target gene expression. Some ligands of TFRs in actinomycetes and their regulatory effects have been identified and studied; however, regulatory mechanisms of the TetR family in the lincomycin-producing *Streptomyces lincolnensis* remain poorly understood.

Results In this study, we found that AbrT (SLCG_1979), a TetR family regulator, plays a pivotal role in regulating lincomycin production and morphological development in *S. lincolnensis*. Deletion of *abrT* gene resulted in increased lincomycin A (Lin-A) production, but delayed mycelium formation and sporulation on solid media. AbrT directly or indirectly repressed the expression of lincomycin biosynthetic (*lin*) cluster genes and activated that of the morphological developmental genes *amfC*, *whiB*, and *ftsZ*. We demonstrated that AbrT bound to two motifs (5′-CGC GTACTCGTA-3′ and 5′-CGTACGATAGCT-3′) present in the bidirectional promoter between *abrT* and *SLCG_1980* genes. This consequently repressed *abrT* itself and its adjacent gene *SLCG_1980* that encodes an arabinose efflux permease. D-arabinose, not naturally occurring as L-arabinose, was identified as the effector molecule of AbrT, reducing its binding affinity to *abrT-SLCG_1980* intergenic region. Furthermore, based on functional analysis of the AbrT homologue in *Saccharopolyspora erythraea*, we inferred that the TetR family regulator AbrT may play an important role in regulating secondary metabolism in actinomycetes.

Conclusions AbrT functions as a regulator for governing lincomycin production and morphological development of *S. lincolnensis*. Our findings demonstrated that D-arabinose acts as a ligand of AbrT to mediate the regulation of lincomycin biosynthesis in *S. lincolnensis*. Our findings provide novel insights into ligand-mediated regulation in antibiotic biosynthesis.

Keywords TetR family regulator, *Streptomyces lincolnensis*, D-arabinose, Antibiotic biosynthesis, Ligand

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Background

Actinomycetes are prolific producers of secondary metabolites, making them valuable sources for natural drug development [1, 2]. Given the prevalence of drug-resistant bacteria, the development of new drugs and high-yield strains are the primary goals in the exploitation of actinomycete drug resources [3]. Innovative metabolic engineering and synthetic biology tools have facilitated the discovery and overproduction of natural products in actinomycetes [4, 5]. Engineering modifications of actinomycetes predominantly involve combining genetic and metabolic engineering techniques with various strategies, such as increasing or decreasing the expression of regulatory factors, enhancing precursor supply, reducing competitive pathways, and boosting the quantity and activity of rate-limiting enzymes [6, 7]. Therefore, understanding the intricate metabolic networks in actinomycetes is crucial for optimising antibiotic production.

Lincomycin, a lincosamide antibiotic produced by *Streptomyces (S) lincolnensis*, is mainly used in clinical settings to treat gram-positive bacterial associated infections [8]. Among the lincomycin biosynthetic gene (*lin*) cluster of *S. lincolnensis*, 29 genes are organized into 13 transcriptional units, spanning over 35 kb of DNA [9–11]. Research on multiple types of transcription factors (TFs) in *S. lincolnensis* has elucidated the intricate mechanisms underlying the molecular regulation of lincomycin biosynthesis [11–13]. The cluster-situated regulatory factor (LmbU) promotes the biosynthesis of lincomycin, while the N-terminal of LmbU includes an auto-inhibitory domain, inhibiting the DNA-binding activity of LmbU [10, 13, 14]. Additionally, LmrC exhibits dual functionality in drug resistance and regulation and can affect the expression of LmbU through antibiotic signal transduction, thereby promoting lincomycin biosynthesis [9]. AdpA positively regulates lincomycin biosynthesis and affects morphological differentiation [15]. The GntR family transcriptional regulator (LcbR1) inhibits lincomycin biosynthesis by indirectly repressing the expression of *lin* cluster genes [16]. SLCG_7083, another major regulator that features a PAS domain, has been shown to promote glucose utilisation, slow mycelial growth, and affect sporulation in *S. lincolnensis* [12]. We have previously demonstrated that the TetR family transcriptional regulator (TFR) SLCG_2919 binds to all promoters within the *lin* cluster to inhibit lincomycin synthesis [11]. The Lrp family regulator (SLCG_ Lrp) can positively regulate lincomycin biosynthesis, with arginine and phenylalanine acting as ligands [17, 18]. While these regulatory factors have been shown to directly or indirectly influence lincomycin biosynthesis and morphological differentiation, a deeper understanding of the regulatory network of lincomycin biosynthesis remains lacking, especially in ligand-mediated molecular regulation.

TFRs are widely distributed in bacteria and archaea as crucial regulators controlling diverse cellular processes [19, 20]. These regulators undergo conformational changes induced by small-molecule ligands, thereby inhibiting or promoting them to control target gene expression [21, 22]. TFRs have numerous ligands, including carbohydrates, proteins, fatty acids and their derivatives, and metal ions [20, 23]. Hence, TFRs regulate several physiological processes, from basic metabolism to quorum sensing and secondary metabolism [23]. Some TFR ligands in actinomycetes and their regulatory effects have been identified and investigated [23]. However, the regulatory role of TFR-responsive ligands in controlling lincomycin synthesis in *S. lincolnensis* remains unknown.

This study reveals the multifaceted role of AbrT (**A**ra**b**inose-**r**esponsive **T**etR type regulator), a TFR, in the regulation of lincomycin production and morphological differentiation. AbrT acts as a repressor of lincomycin production while acting as an activator to regulate morphological differentiation. AbrT could bind to the bidirectional promoter between *acrT* and *SLCG_1980* to transcriptionally inhibit *abrT* and *SLCG_1980*. Then, we identified two motifs within *abrT-SLCG_1980* intergenic region, crucial for AbrT recognition. Furthermore, we demonstrated that D-arabinose acts as a ligand of AbrT, reducing its binding affinity to *abrT-SLCG_1980* intergenic region. AbrT homologue in the actinomycete *Saccharopolyspora erythraea* was also found to inhibit the erythromycin production, suggesting that AbrT may play an important role in regulating secondary metabolism in actinomycetes.

Methods

Strains, plasmids, and growth conditions

Table 1 lists the bacterial strains and plasmids used in this study. *Escherichia* is usually cultured in liquid Luria Bertani (LB) medium or solid LB medium, maintained at 37℃ for approximately 8–12 h [11]. *S. lincolnensis* LCGL and its derivatives were incubated on a solid MGM medium at 30℃ for 7 days [11]. *Sac. erythraea* A226 and its derivatives were cultured on a solid R3M medium at 30℃ for sporulation and protoplast regeneration [6]. Cell growth was measured in liquid TSBY medium (3% tryptone soy broth, 0.5% yeast extract, 10.3% sucrose, with/without apramycin or thiostrepton) [17]. Liquid minimal medium (MM) (50% glucose, 0.5 g/l L-asparagine, 0.5 g/l K_2HPO_4, 0.2 g/l $MgSO_4{\cdot}7H_2O$, 0.01 g/l $FeSO_4{\cdot}7H_2O$) supplemented with 1 mM glutamine, glutamate, $NaNO_2$ or $NaNO_3$ was used for the detection of fluorescence reporting system in *S. lincolnensis* [17]. Yeast Malt Glucose (YMG) medium (10 g/l malt extract, 4 g/l yeast

Table 1 Bacterial strains and plasmids used in this study

Strains and plasmids	Description	Reference
E. coli		
DH5α	F recA lacZM15	[24]
BL21 (DE3)	F^{-}ompThsdSB($rB^{-}mB^{-}$) dcmgalλ(DE3)	Novagen
S. lincolnensis		
LCGL	LC-G derivative with artificially integrated $attB^{\Phi C31}$site	[25]
Δ*abrT*	LCGL derivative with *abrT gene* deleted	This study
Δ*abrT*/pIB139	Δ*abrT* carrying pIB139	This study
Δ*abrT*/pIB139-*abrT*	Δ*abrT* carrying pIB139-*abrT*	This study
LCGL/pIB139	LCGL carrying pIB139	This study
LCGL/pIB139-*abrT*	LCGL carrying pIB139-*abrT*	This study
LA219X	a lincomycin high-yield strain with artificially integrated $attB^{\Phi C31}$site	[25]
LA219XΔ*abrT*	LA219X derivative with *abrT gene* deleted	This study
Sac. erythraea		
A226	An erythromycin low-producer	CGMCC 8279
Δ*SACE_5812*	A226 derivative with *SACE_5812 gene* deleted	This study
Δ*SACE_5812*/pIB139	Δ*SACE_5812* carrying pIB139	This study
Δ*SACE_5812*/pIB5812	Δ*SACE_5812* carrying pIB139-*5812*	This study
A226/pIB139	A226 carrying pIB139	This study
A226/pIB5812	A226 carrying pIB139-*5812*	This study
Plasmids		
pUCTSR	pUC18 derivative containing a 1.36-kb fragment of a thiostrepton resistance gene in *Bam*HI/*Sma*I sites	[26]
pUCTSRΔ*abrT*	pUCTSR derivative containing two 1.5-kb fragments, the upstream and downstream regions of *abrT*	This study
pUCTSRΔ*5812*	pUCTSR derivative containing two 1.5-kb fragments, the upstream and downstream regions of *SACE_5812*	This study
pKC1139	*ori* (pSG5), *aac(3)IV*, *lacZ*	[27]
pKC1139-Δ*abrT*	pKC1139 derivative for *abrT* gene deletion	This study
pIB139	øC31 *attP-int* locus, *acc(3)IV*, *oriT*, *PermE** promoter	[28]
pIB139-*abrT*	pIB139 carrying an extra *abrT* for the gene complementation	This study
pIB139-*5812*	pIB139 carrying an extra *SACE_5812* for the gene complementation	This study
pET28a	T7 promoter, His-tag, *kan*	Novagen
pET28a-abrT	pET28a-derived plasmid carrying *abrT*	This study
pET28a-5812	pET28a-derived plasmid carrying *SACE_5812*	This study

extract, 4 g/l glucose) was used for the determination of *S. lincolnensis* biomass [11].

Primers

Supplementary Table S1 lists all primers used in this study.

Gene inactivation, complementation, and overexpression

Genomic DNA from *S. lincolnensis* LCGL was used as a template for PCR amplification using primers abrT-P1/P2 and abrT-P3/P4 to generate a 1504 bp upstream and 1515 bp downstream fragment, respectively. Subsequently, the plasmid pUCTSR and upstream fragment of *abrT* were digested with the restriction enzymes *Hin*dIII and *Xba*I, followed by ligation to obtain plasmid pUCTSR-*abrT*-up, confirmed by enzyme digestion. Plasmid pUCTSR-*abrT*-up and the downstream fragment of *abrT* were digested with the restriction enzymes *Eco*RI and *Kp*nI, followed by ligation. The resulting plasmid pUCTSR-Δ*abrT* was verified through PCR and enzyme digestion. The plasmid pUCTSR-Δ*abrT* was digested with *Hind*III and *Eco*RI and cloned into pKC1139 to obtain pKC1139-Δ*abrT*. Finally, pKC1139-Δ*abrT* was introduced into the protoplasts of *S. lincolnensis* LCGL. By the homologous chromosomic recombination, a 552-nt fragment of the *abrT* gene was replaced by thiostrepton resistance gene (*tsr*) in *S. lincolnensis* LCGL. The desired thiostrepton-resistant mutant, named Δ*abrT*, was confirmed by PCR using the primers abrT-P5/P6. The mutant strain LA219XΔ*abrT* was constructed in the high-yielding strain *S. lincolnensis* LA219X using the same method described above.

abrT was amplified using PCR and the genomic DNA of *S. lincolnensis* LCGL as a template. The amplified fragment was inserted between the *Nde*I and *Xba*I restriction sites of pIB139 to construct complementation plasmid pIB139-*abrT* with PermE* promoter. Subsequently, Δ*abrT* was transformed with pIB139 and pIB139-*abrT* using PEG-mediated protoplast transformation. Initial screening was conducted using apramycin, and PCR identification targeting the apramycin resistance gene was performed to obtain the empty vector

control strain Δ*abrT*/pIB139 and the complementation strain Δ*abrT*/pIB139-*abrT*. Using the method described above, pIB139-*abrT* was transformed into *S. lincolnensis* LCGL, and the constitutive expression strain LCGL/pIB139-*abrT* of *abrT* was identified using PCR.

Sac. erythraea mutants were constructed as previously described [23]. Using *Sac. erythraea* A226 genomic DNA as a template, two 1.5-kb DNA fragments flanking the *SACE_5812* were amplified with PCR using the primer pairs 5812-P1/P2 and 5812-P3/P4. The two PCR products were respectively digested with *Hind*III/*Xba*I and *Kpn*I/*Eco*RI, and ligated into the corresponding sites of pUCTSR to obtain pUCTSR-5812 [23]. The pUCTSR-5812 plasmid was then introduced into *the Sac. erythraea* A226. A 354 bp fragment within SACE_5812 was replaced by *tsr* through homologous recombination of linear fragments in A226. Confirmation of the desired thiostrepton-resistant mutant, named Δ*SACE_5812*, was achieved using the primers 5812-P5/P6 through PCR. Similarly, the Δ*SACE_5812*/pIB5812 complementation strain and A226/pIB1395812 constitutive expression strain were obtained.

Antibiotic fermentation and measurement

An agar piece of approximately 1 cm^3 sample of *S. lincolnensis* LCGL and its derivatives, grown on spore-producing medium MGM, was taken and inoculated into seed medium. After culturing at 30℃ and 240 rpm for 48 h, the sample was transferred to 30 mL of fermentation medium at a 10% inoculation rate and cultured at 30℃ and 240 rpm for 7 days. Upon fermentation completion, 2 mL of the bacterial suspension was transferred to an Eppendorf tube and centrifuged at 12,000 rpm for 10 min. Subsequently, 200 µL of the supernatant was transferred to a new Eppendorf tube, and 800 µL of anhydrous ethanol was added and mixed. The mixture was allowed to stand for 5 min and centrifuged at 12,000 rpm for 10 min. The supernatant was then collected and filtered through a 0.22 µm organic filter membrane. Prepared filtrate liquid samples were subjected to High-performance liquid chromatography (HPLC) to detect Lin-A production using an Extend-C18 chromatographic column (5 µm, 150×4.6 mm). The mobile phase for equilibration comprised 60% methanol and 40% 5 mmol/L ammonium acetate (pH 9.0), with a flow rate of 0.5 mL/min and a detection wavelength of 214 nm. Flask fermentation by *Sac. erythraea* A226 and its derivatives were prepared as described [23].

The mutant Δ*abrT* and original strain LCGL were inoculated into YMG medium, respectively, and fermented at 30℃ for 7 days. During the fermentation period, the samples were weighed daily to measure their biomass [17].

Total RNA extraction and quantitative real-time PCR (qRT-PCR)

RNA was extracted from *S. lincolnensis*, and gene expression was measured as described by Xu et al. [17].

Protein expression and purification

The genomic DNA from *S. lincolnensis* LCGL was used as a template, and the *abrT* fragment was amplified using PCR and primers abrT-P7 and abrT-P8. After digestion with *Nde*I and *Hind*III, the *abrT* fragment was cloned into the pET28a vector, generating the protein expression vector pET28a-abrT. This vector was then introduced into *Escherichia coli* (*E. coli*) BL21 (DE3) cells. The transformed strain was cultured in 50 mL of liquid LB medium at 37 °C until its optical density at 600 nm (OD_{600}) reached 0.4–0.6. Subsequently, IPTG was added to a final concentration of 0.5 mM, and expression was induced for 20 h at 16 °C. After induction, the strain was briefly cooled on ice and centrifuged at 6000 rpm for 10 min. The collected cells were resuspended in a lysis buffer (20 mM Tris, 500 mM NaCl, pH8.0) and disrupted via ultrasonication. The lysate was centrifuged at 4000 rpm for 30 min, and the His_6-tagged AbrT protein in the supernatant was recovered using a Ni^{2+}-nitrilotriacetic acid (NTA) spin column (Bio-Rad). The AbrT protein was subsequently eluted from the column with 500 mM imidazole. The purity of the fusion protein was assessed via SDS-PAGE, and the protein concentration was determined using a BCA assay.

Electrophoretic mobility shift assays (EMSAs)

EMSAs for AbrT and SACE_5812 protein were conducted as previously described [17]. To investigate potential effectors of AbrT, D-arabinose and L-arabinose were separately added to AbrT protein and probe DNA in a 20 µL reaction mixture, respectively. The samples were then analysed through PAGE, as described by Xu et al. [17].

DNase I footprinting assay

To identify the binding site of AbrT in the intergenic region between *abrT* and *SLCG_1980*, we performed a DNase I footprint analysis following the methods outlined by Xu et al. [11].

Fluorescence reporting system experiment

To construct the reporter plasmid, we amplified the *abrT-SLCG_1980*-int fragment using the LCGL genome as a template. The resulting fragments were cleaved using *Hind*III and *Xba*I. Simultaneously, *egfp* fragments were amplified using the pUPW-EGFP plasmid as a template and digested with *Xba*I and *Bam*HI. Subsequently, the cleaved *abrT* and *egfp* fragments were cloned into the pKC1139 plasmid, resulting in the fluorescent reporter system control plasmid, pKC-DE. The plasmid

pKC-AbrT-DE was obtained using the same method. These plasmids were used to transform *E. coli* DH5α to obtain the corresponding strains. In a 48-well cell culture plate containing 500 μL LB, 5 μL of transformed strains were inoculated. After adding ligands at various concentration gradients, the bacteria were cultured at 37 °C until the OD_{600} reached 0.8–1.0. The optical density (OD) values of the bacterial solution at 600 nm were measured using a multifunctional microplate reader, and the fluorescence signal values of the corresponding samples were detected using an excitation filter at 485 nm and an emission filter at 535 nm on a high-end SpectraMax Paradigm.

Similarly, the plasmids pKC-DE and pKC-AbrT-DE were transformed into the *S. lincolnensis* Δ*abrT* to obtain the corresponding strains. These transformed strains were then transferred to a 24-well cell culture plate containing 1 mL MM medium with different concentration ligands and cultured until their OD_{600} reached 1.2–1.3. The detection was conducted using the method described above.

Statistical analysis

All data in this study were collected in triplicate and are presented as means ± standard deviation [29]. Significance was analysed using an unpaired two-tailed Student's *t*-test, with $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$.

Results and discussion

AbrT negatively regulates lincomycin biosynthesis

AbrT contains 714 nucleotides (nt) and encodes a TetR family protein consisting of 237 amino acids with an unresolved regulatory function in *S. lincolnensis*. To elucidate the relationship between AbrT and lincomycin production, we disrupted the *abrT* gene with *tsr* replacement in *S. lincolnensis* LCGL (Fig. 1a) and confirmed the

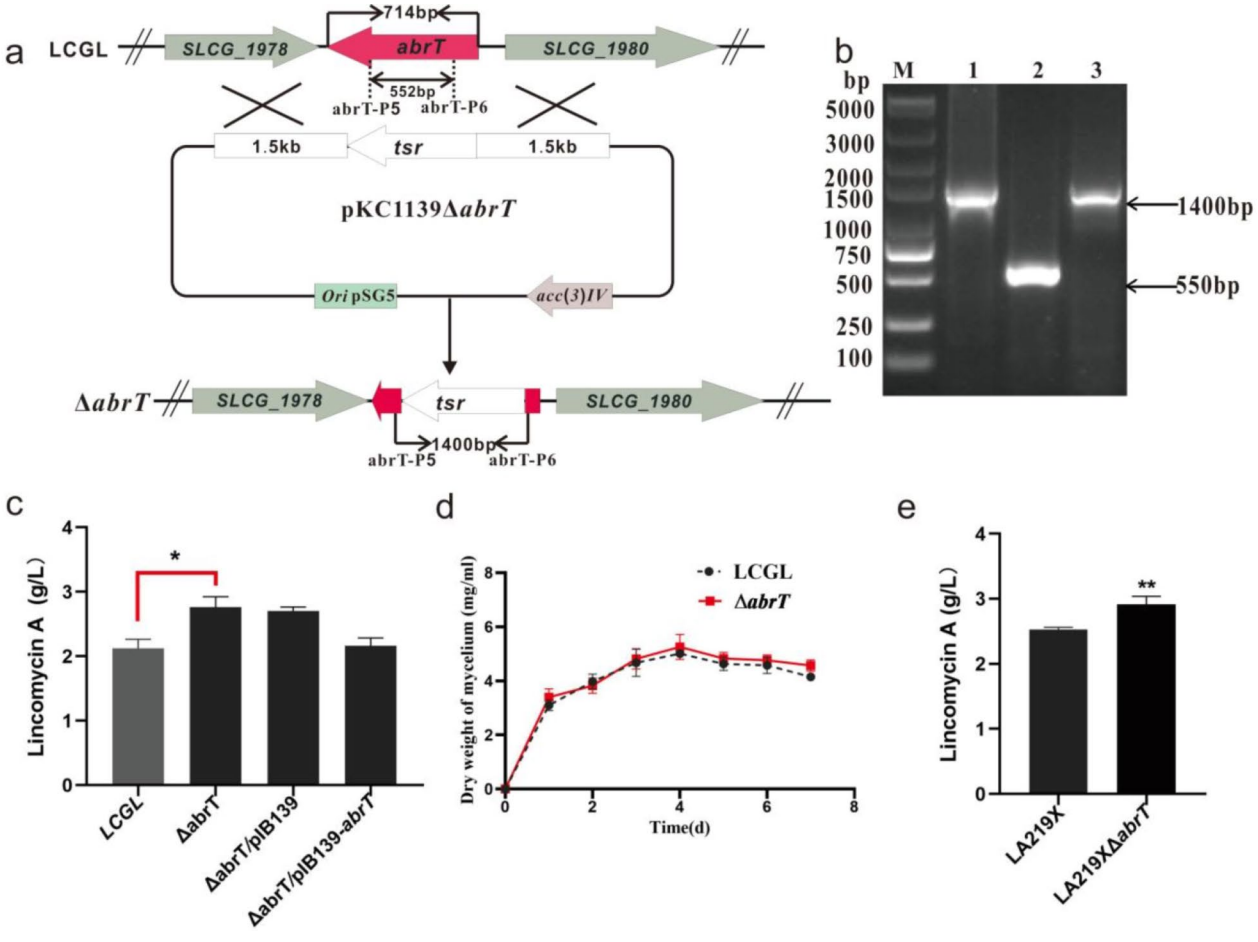

Fig. 1 AbrT negatively regulates lincomycin production in *S. lincolnensis*. (**a**) Schematic representation of *abrT* gene deletion via homologous recombination in *S. lincolnensis* LCGL. (**b**) PCR confirmation of the *abrT* gene deletion mutant using primers abrT-P5/P6. Lanes: M, 5000 bp DNA ladder; 1, positive control, 1400 bp amplified from pKC1139Δ*abrT*; 2, negative control, 550 bp amplified from LCGL; 3, sample, 1400 bp amplified from mutant Δ*abrT*. (**c**) The Lin-A production in LCGL, Δ*abrT*, Δ*abrT*/pIB139, and Δ*abrT*/pIB139-*abrT*. (**d**) Growth curves of LCGL and Δ*abrT* in the liquid fermentation medium, with dry weights of mycelia measured. (**e**) The Lin-A production in LA219X and LA219X/Δ*abrT*. Mean values of three replicates are shown, with the standard deviation indicated by error bars. $^{*}p<0.05$, $^{**}p<0.01$

resulting mutant Δ*abrT* using PCR (Fig. 1b). Fermentation and HPLC analyses revealed that lincomycin A (Lin-A) production in Δ*abrT* was approximately 28% higher than that in LCGL (Fig. 1c). The Lin-A production could be lowered by complementing the Δ*abrT* mutant with pIB139-*abrT*, confirming that the increased Lin-A production was attributed to *abrT gene* deletion (Fig. 1c). Furthermore, we analysed the growth curve of LCGL and Δ*abrT* strains in fermentation medium by measuring mycelium dry weight and found that *abrT* deletion had no significant effect on cell growth (Fig. 1d). These findings indicate that AbrT negatively regulates Lin-A production in *S. lincolnensis*.

To examine the role of AbrT as a negative regulator of lincomycin production in *S. lincolnensis*, *abrT* was deleted from the high-yield strain *S. lincolnensis* LA219X. When cultured in a 30 mL industrial fermentation medium for 7 days, the resulting *S. lincolnensis* LA219XΔ*abrT* (2.91 g/L) exhibited a 16% higher increment in Lin-A production higher than that of LA 219X (2.52 g/L) (Fig. 1e).

AbrT represses the *lin* cluster genes

To explore the regulatory role of AbrT, we utilised qRT-PCR to compare the transcripts of genes within the *lin* cluster between LCGL and Δ*abrT* at 24 h and 48 h. The endogenous *rpoD* gene, which exhibits a constant transcriptional level in LCGL and its derivatives, was used as an internal control to normalize samples. At 24 h, only the transcription levels of structural gene *lmbA*, bi-functional gene *lmrC*, and regulatory gene *lmbU* in Δ*abrT* were 1.7-, 1.5-, and 1.4-fold higher, respectively, than those in LCGL (Fig. 2a). While at 48 h, structural genes *lmbC*, *lmbE*, *lmbG*, *lmbV*, resistance gene *lmrA*, and *lmrC* in Δ*abrT* were 1.5-, 1.4-, 3.8-, 1.5-, 1.8-, and 1.4-fold higher, respectively, than those of LCGL (Fig. 2a). These findings indicate that disrupting *abrT gene* increased Lin-A production by enhancing the transcription of *lin* genes.

To determine whether AbrT could directly regulate the transcription of these genes, we expressed His_6-tagged AbrT protein in *E. coli* BL21 (DE3) (Fig. S1) and examined its affinities with the promoter regions of *lmbA*, *lmbC-lmbD*, *lmbE*, *lmbJ-lmbK*, *lmbV-lmbW*, *lmrA*, *lmrC*, and *lmbU*. EMSAs showed that the purified His_6-tagged AbrT bound to the promoter regions of *lmrC* and *lmbU* when different concentrations of His_6-AbrT were added (Fig. 2b), These findings indicate that AbrT directly or indirectly repressed five structural genes (*lmbA*, *lmbC*, *lmbE*, *lmbG*, and *lmbV*), one resistance gene (*lmrA*), one bifunctional gene (*lmrC*), and one regulatory gene (*lmbU*) in *S. lincolnensis*.

AbrT is involved in the morphological differentiation of *S. lincolnensis*

Previous studies have shown that the deletion of *bldA* [30], *bldD* [31], *adpA* [15], and *ramR* [32] delays aerial mycelium formation and sporulation in *S. lincolnensis*. To determine AbrT contributions to morphological differentiation, we inoculated LCGL and Δ*abrT* on spore-producing culture medium for 48, 72, and 96 h. The Δ*abrT* exhibited a markedly delayed formation of mycelia and spores compared with the LCGL. This morphological phenotype was restored in Δ*abrT*/pIB139-*abrT* (Fig. 3a). These findings indicate that knocking out *abrT* retards aerial mycelium formation and sporulation in *S. lincolnensis*.

Therefore, we conducted qRT-PCR for several key genes involved in morphological differentiation, including *amfC* (SLCG_2911, encoding an aerial mycelium formation protein) [33], *whiB* (SLCG_3418, encoding a sporulation regulatory protein) [34] and *ftsZ* (SLCG_6003, encoding a cell division protein) [35]. The transcriptional levels of *ftsZ*, *whiB*, and *amfC* were lower in Δ*abrT* than in LCGL (Fig. 3b). Furthermore, we observed direct binding of AbrT to the promoter regions of these three genes (Fig. 3c), suggesting that AbrT positively regulates morphological differentiation by directly activating the transcription of *ftsZ*, *whiB*, and *amfC*. Collectively, these findings indicate that AbrT exhibits opposing regulatory effects, inhibiting lincomycin biosynthesis while stimulating morphological development in *S. lincolnensis*.

AbrT represses the transcription of *abrT* and its adjacent gene *SLCG_1980*

Following the regulatory paradigm of SLCG_2919 [11], we predicted that AbrT may regulate the expression of its gene and its adjacent divergently transcribed gene, *SLCG_1980*, which encodes an arabinose efflux permease. We assessed the transcription levels of *abrT* and *SLCG_1980* using qRT-PCR. Analysis of the remaining 100-nt 5'-region of the *abrT* gene revealed a 16-fold increase in *abrT* transcription in Δ*abrT* compared with in LCGL at 24 h (Fig. 4a). Similarly, the expression level of *SLCG_1980* in Δ*abrT* strikingly increased by 500-fold at 24 h. At 48 h, the expression levels of *abrT* and *SLCG_1980* increased by 8-fold and 17-fold, respectively (Fig. 4a). These findings indicate that AbrT acts as a repressor of its gene and the adjacent gene *SLCG_1980*.

EMSA demonstrated a clearly shifted band formed when AbrT was incubated with *abrT-SLCG_1980* intergenic region (*abrT-SLCG_1980-int*), confirming direct binding of AbrT to *abrT-SLCG_1980* intergenic region (Fig. 4b). Previous studies and bioinformatics analyses (http://www.fruitfly.org/seq_tools/promoter.html) predicted that the DNA fragment between *abrT* and

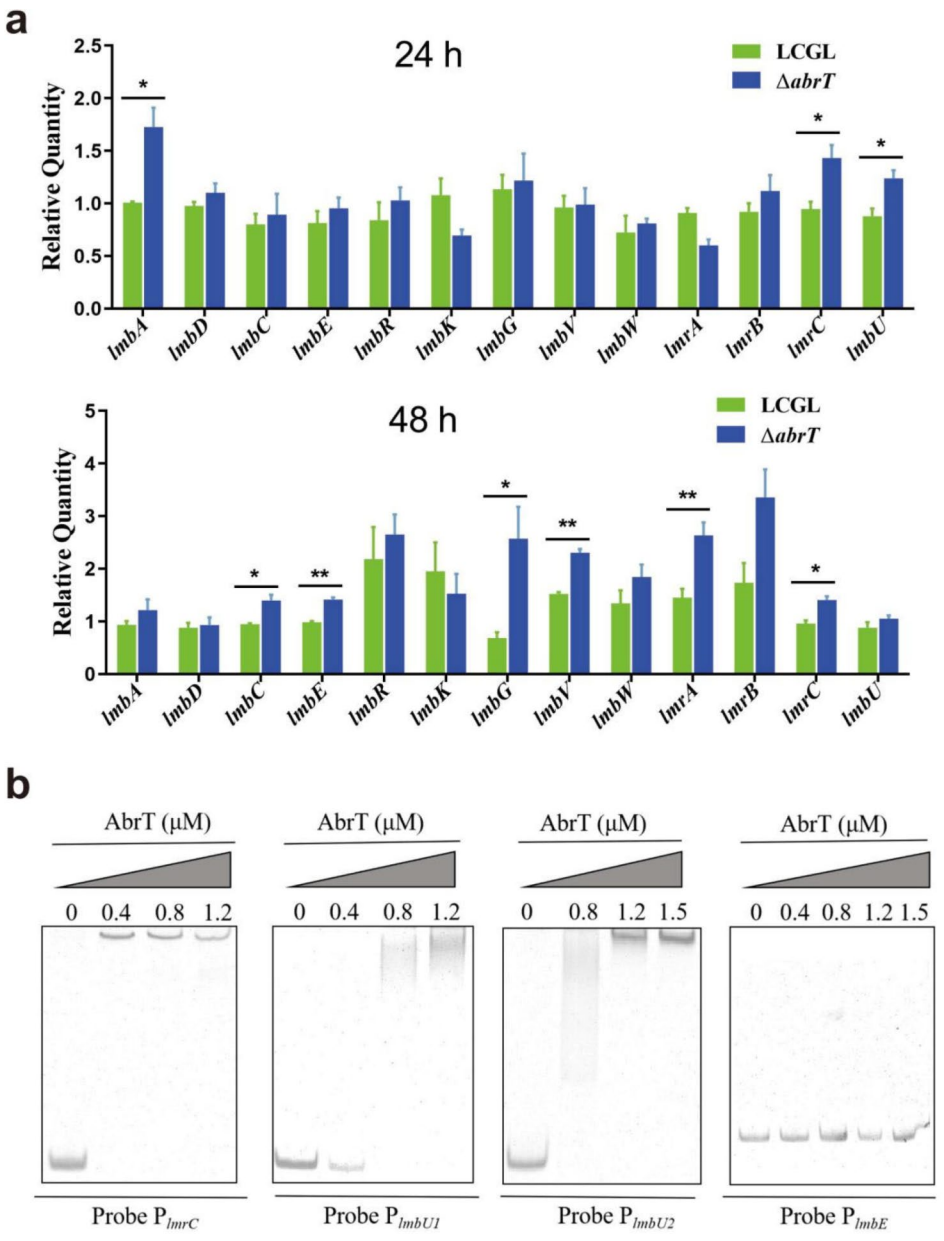

Fig. 2 AbrT-mediated regulation to the *lin* cluster genes. **(a)** Quantitative transcription levels of *lin* cluster genes in LCGL and Δ*abrT* cultured for 24 and 48 h in the fermentation medium. Mean values of three replicates are shown, with the standard deviation indicated by error bars. $*p < 0.05$, $**p < 0.01$. (**b**) EMSAs show the interaction of His_6-AbrT with the promoter regions of *lmrC* (20 nM DNA probes), *lmbU* (20 nM DNA probes), and *lmbE* (40 nM DNA probes)

SLCG_1980 is a bidirectional promoter controlling the expression of *abrT* and *SLCG_1980* (Fig. S2).

Determination of AbrT-binding sites within abrT-SLCG_1980 intergenic region

To determine the AbrT binding site in the promoter regions of *abrT* and *SLCG_1980*, we conducted a DNase I footprinting assay using Fam-labelled DNA fragments. Two binding sites for AbrT were identified: site a (5′-CG CGTACTCGTA-3′) and site b (5′-CGTACGATAGCT-3′) (Fig. 5a). To verify the importance of these motifs for AbrT binding, EMSAs were performed using the *abrT-SLCG_1980-int* (N), a single-site mutated DNA fragment (M1 or M2), and a double-site mutated DNA fragment (M3) (Fig. 5b). In contrast to N, probes M1 and M2 displayed only a single shifted band with no shifted band in the gel using probe M3 (Fig. 5c). This indicates that two binding sites are present for AbrT to *abrT-SLCG_1980* intergenic region.

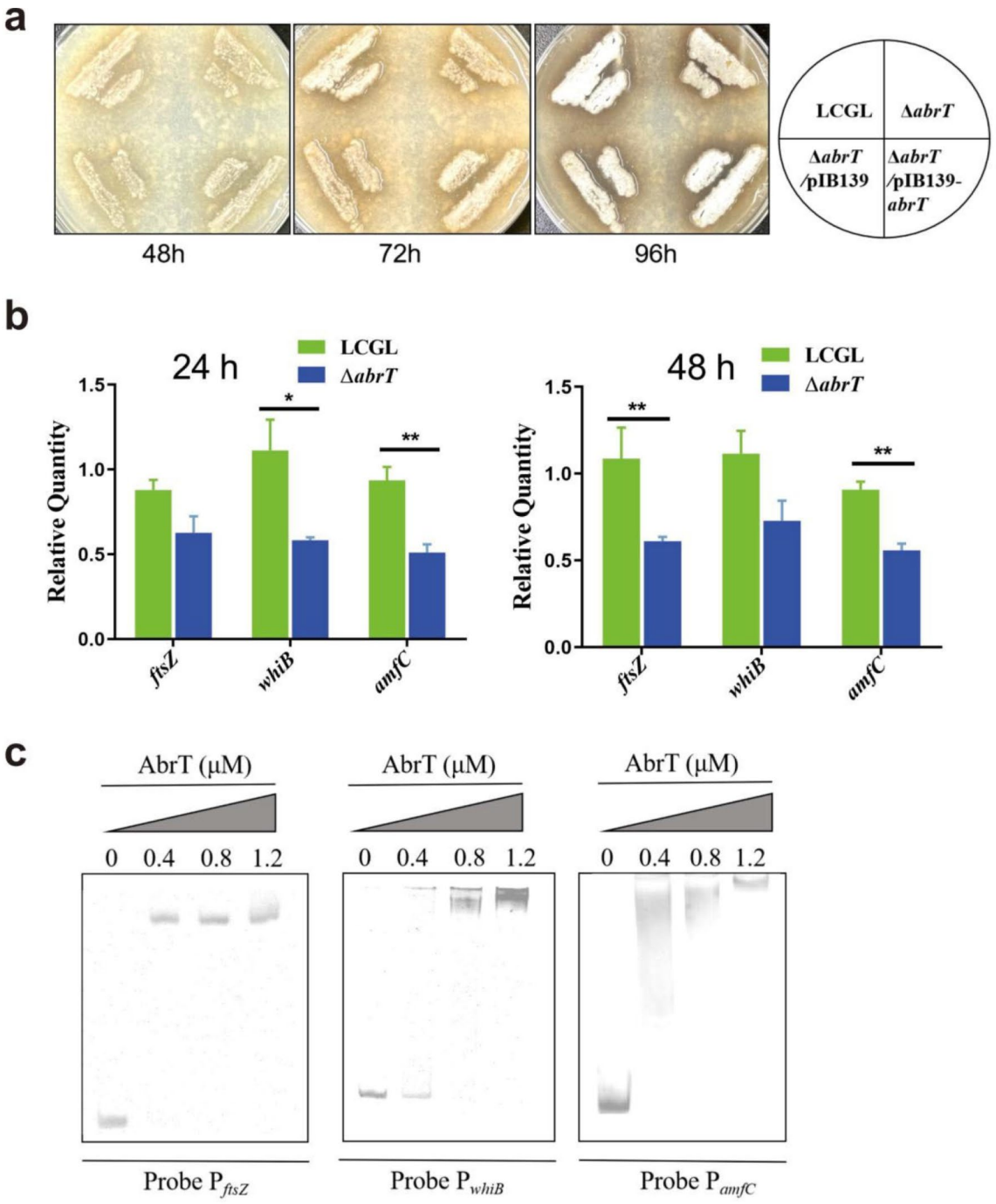

Fig. 3 AbrT positively regulates *S. lincolnensis* morphological differentiation. **(a)** Morphological observation of the original strain LCGL, Δ*abrT*, Δ*abrT*/pIB139, and Δ*abrT*/pIB139-*abrT* on MGM medium for 48, 72, and 96 h. (**b)** qRT-PCR analysis of the genes *ftsZ*, *whiB*, and *amfC* involved in morphological differentiation in LCGL and Δ*abrT* cultured for 24 and 48 h in a fermentation medium. Mean values of three replicates are shown, with the standard deviation indicated by error bars. *$p < 0.05$, **$p < 0.01$. (**c)** EMSAs of AbrT binding to the probes P_{ftsZ} (25 nM), P_{whiB} (60 nM), and P_{amfC} (40 nM) represent the promoters of *ftsZ*, *whiB*, and *amfC*, respectively

AbrT is responsive to D-arabinose in vitro and in vivo

TFRs have various ligands, including carbohydrates, proteins, fatty acids and their derivatives, and metal ions [20, 23]. Given that *SLCG_1980*, the target gene of AbrT, encodes an arabinose efflux permease, exploring whether AbrT regulates *SLCG_1980* in response to arabinose as an effector is essential. To test this hypothesis, EMSAs were performed with AbrT binding to the *abrT-SLCG_1980-int* upon exogenous addition of L-arabinose or D-arabinose. The addition of D-arabinose reduced the

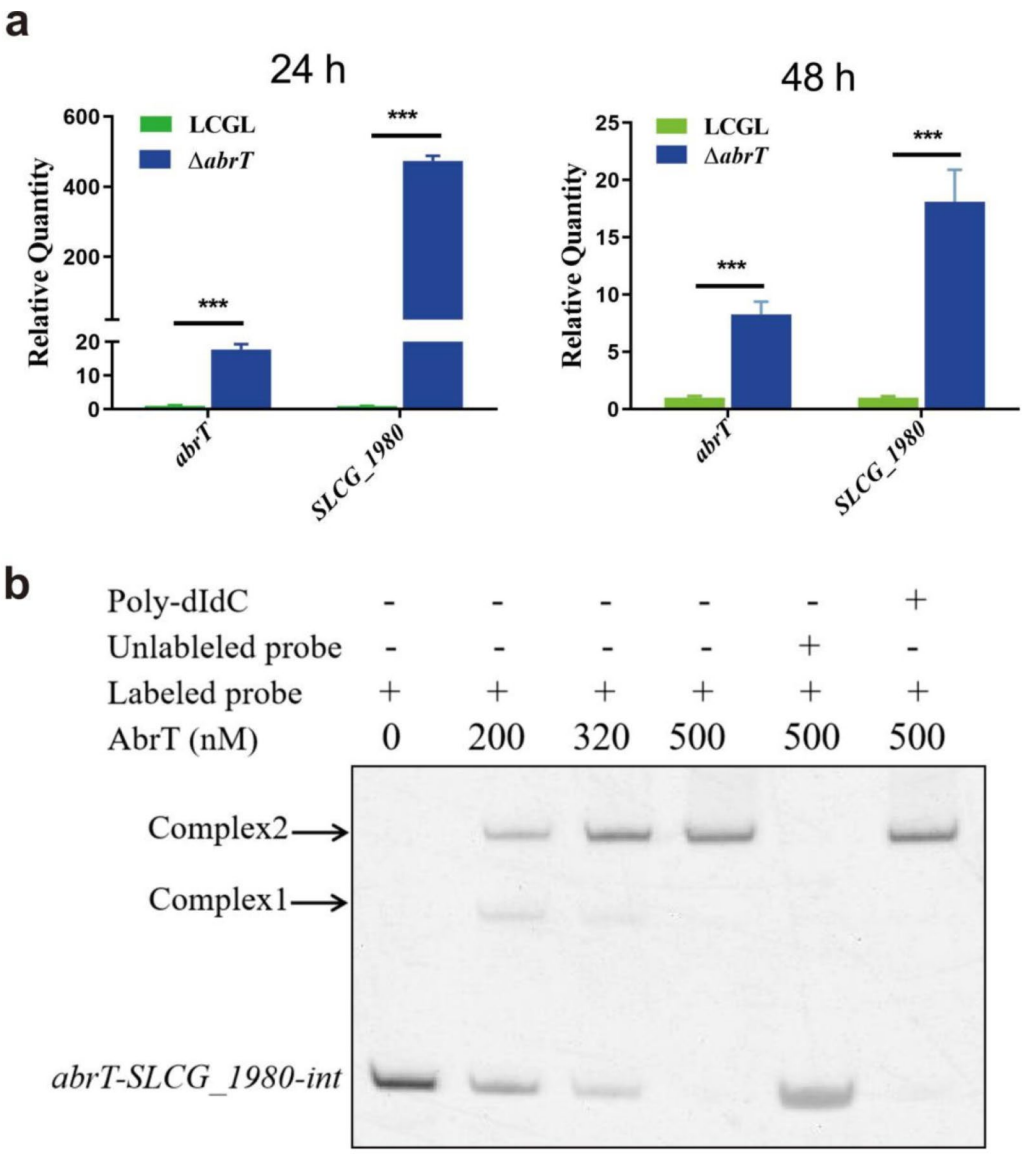

Fig. 4 AbrT represses the transcription of *abrT* and its adjacent gene *SLCG_1980*. **(a)** Transcriptional levels of *abrT* and *SLCG_1980* in LCGL and Δ*abrT* assessed using qRT-PCR analysis at 24 and 48 h. Mean values of three replicates are presented, with the standard deviation indicated by error bars. ***$p < 0.001$. **(b)** EMSA demonstrating the binding of AbrT to *abrT-SLCG_1980-int*. Each lane contained 50 nM DNA probes

binding affinity between AbrT and *abrT-SLCG_1980-int*, suggesting that D-arabinose acts as an effector of AbrT (Fig. 6a).

To determine whether AbrT interacted with D-arabinose in vivo, we constructed a biosensor system in *E. coli* DH5α following our previous method [6]. This system employed two plasmids: pKC-AbrT-DE, which expressed the reporter *egfp* gene, and the *abrT* gene controlled by the bidirectional promoter between *abrT and SLCG_1980* genes ($P_{abrT-1980}$), and pKC-DE, which solely expressed the reporter *egfp* gene under the control of $P_{abrT-1980}$. (Fig. 6b). Upon the addition of 0.5–100 μM D-arabinose, a gradual increase was observed in green fluorescence in DH5α/pKC-AbrT-DE (Fig. 6c). Meanwhile, the addition of L-arabinose in the same concentration range showed no difference in bioluminescence (Fig. 6d). These findings indicate that similar to its in vitro effects, D-arabinose is an effector of AbrT in vivo.

The fluorescent reporter system in *E. coli* has demonstrated that, in vivo, D-arabinose acts as the dissociative ligand of AbrT, binding to $P_{abrT-1980}$, with a minimum effective concentration for allosteric regulation of 1 μmol/L. However, given that the fluorescence reporter system in heterologous hosts cannot reflect the response of AbrT to ligands under physiological conditions, we performed biofluorescence measurements in *S.*

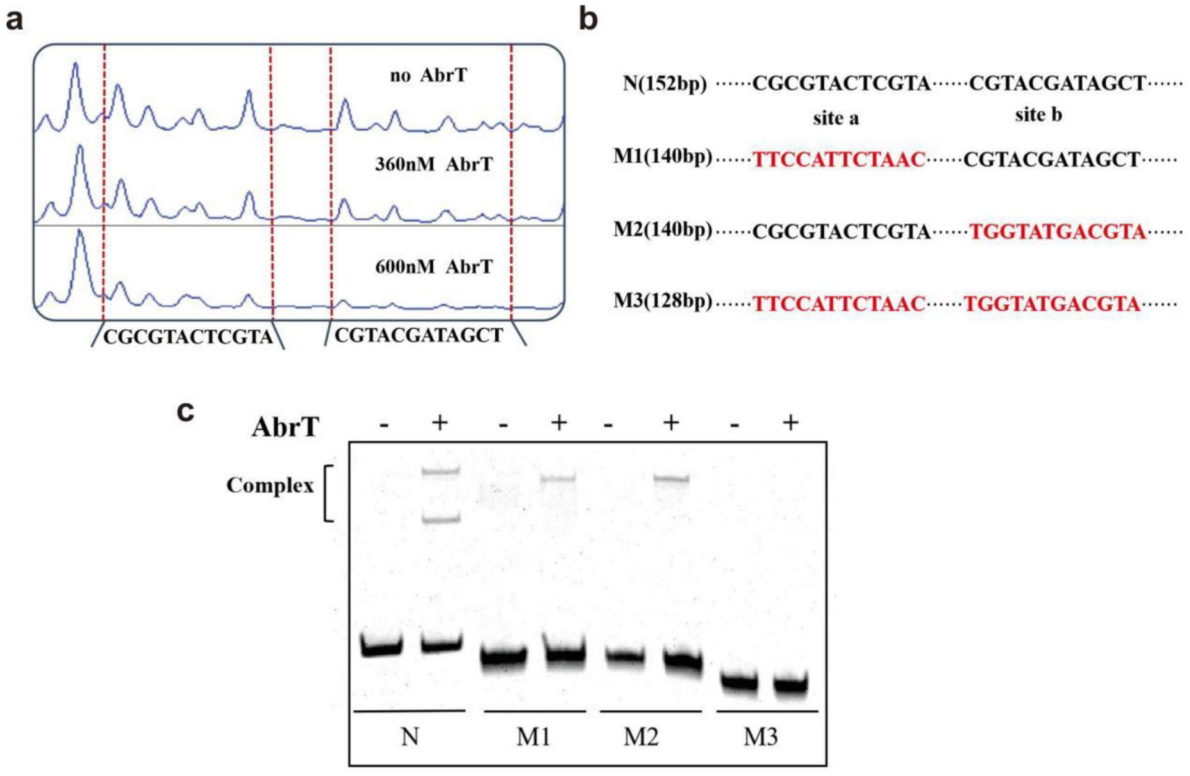

Fig. 5 Determination of the precise binding sites of AbrT. **(a)** DNase I footprinting assay showing AbrT-binding sites within *abrT-SLCG_1980* intergenic region. **(b)** Schematic diagrams of the mutated probes. Probe N, *abrT-SLCG_1980-int*, served as the control; probes M1 had only site a within the 140 bp DNA fragment mutated; M2 had only site b in the 140 bp DNA fragment mutated; M3 had site a and b within the 128 bp DNA fragment mutated. **(c)** EMSAs show AbrT binding to probe N and mutated probes M1, M2, and M3

lincolnensis to explore the physiological state of D-arabinose as a ligand influencing the allosteric regulation of AbrT. To minimise interference of AbrT in *S. lincolnensis* with the fluorescence reporting system, the two plasmids, pKC-DE and pKC-AbrT-DE, were successively introduced into Δ*abrT*. Biofluorescence assays revealed that supplementation of external D-arabinose ranging from 0.1 to 5 μM in Δ*abrT*/pKC-DE did not yield a significant change in fluorescence levels. However, in Δ*abrT*/pKC-AbrT-DE, the fluorescence intensity showed a positive correlation with increasing D-arabinose levels, consistent with the results from *E. coli* (Fig. 6e). This suggests that the physiological concentration of D-arabinose in regulating its function in *S. lincolnensis* is approximately 0.1 μmol/L, with a response sensitivity nearly 10 times greater than observed in *E. coli*.

AbrT homolog plays an important role in the antibiotic biosynthesis

Given the presence of AbrT homologues in antibiotic-producing actinomycetes (Fig. 7a), we explored whether their regulatory functions have commonalities. SACE_5812, a TetR family regulator sharing the highest amino acid identity with AbrT (Fig. 7a), was selected to investigate the erythromycin A (Er-A)-producing actinomycete *Sac. erythraea* [24]. Genetic experiments involving disruption and complementation of *SACE_5812* demonstrated its negative effect on Er-A production (Fig. 7b). Using qRT-PCR and EMSAs, we found that SACE_5812 directly repressed the expression of *SACE_5813*, *eryAI* (encoding polyketide synthase I), and *ermE* (encoding rRNA methyltransferase), suggesting its direct regulation of the erythromycin biosynthesis gene cluster and its adjacent gene in *Sac. erythraea* A226 (Fig. 7c and d). Those results are consistent with the regulatory function of AbrT in *S. lincolnensis*. These findings suggest that AbrT and its homologs may play the important role in regulating secondary metabolism in actinomycetes.

Conclusions

The findings of this study demonstrated the regulatory role of the TetR family protein AbrT in lincomycin production by repressing the expression of *lin*

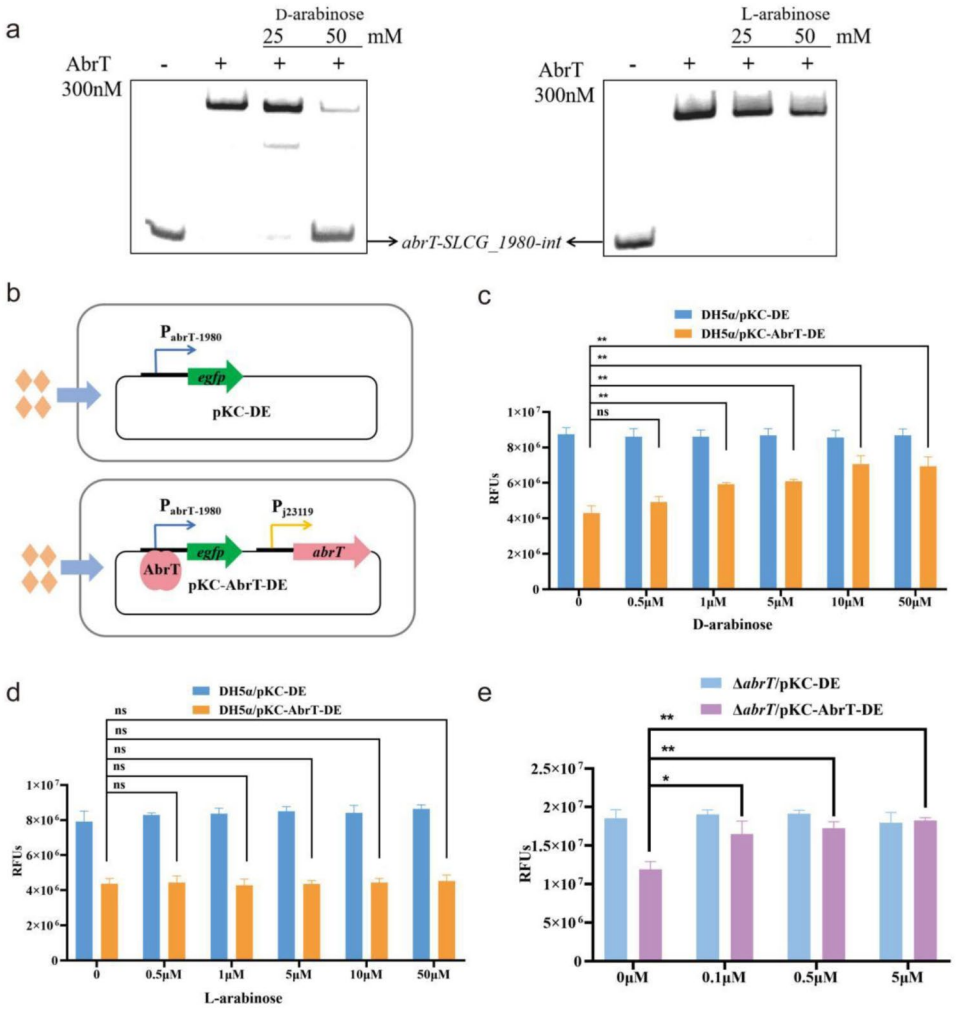

Fig. 6 D-arabinose induces the dissociation of AbrT from $P_{abrT-1980}$. **(a)** EMSAs of AbrT with D-arabinose or L-arabinose. All arabinose concentrations are expressed in mM. **(b)** An illustration of the reporter plasmids. The addition of the respective arabinose effectors affects *egfp* expression in the reporter system. **(c)** Detection of relative fluorescence units (RFUs) after adding D-arabinose in *E. coli* DH5α/pKC-AbrT-DE and DH5α/pKC-DE. **(d)** Detection of RFUs after adding L-arabinose in *E. coli* DH5α/pKC-AbrT-DE and DH5α/pKC-DE. **(e)** Detection of RFUs after adding D-arabinose in Δ*abrT*/pKC-AbrT-DE and Δ*abrT*/pKC-DE. The Mean values of three replicates are shown, with standard deviation indicated by error bars. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

cluster genes in *S. lincolnensis.* AbrT directly inhibited the expression of its gene and that of the neighbouring gene, *SLCG_1980*, through two DNA-binding sequences. Our study reveals that D-arabinose acts as a ligand to modulate lincomycin biosynthesis in *S. lincolnensis*. Furthermore, functional analysis of an AbrT-homologue protein in *Sac. erythraea* suggested that AbrT may play an important role in regulating secondary metabolism in actinomycetes. Moreover, AbrT affected mycelial growth and morphological development by directly activating the expression of *amfC*, *whiB*, and *ftsZ*.

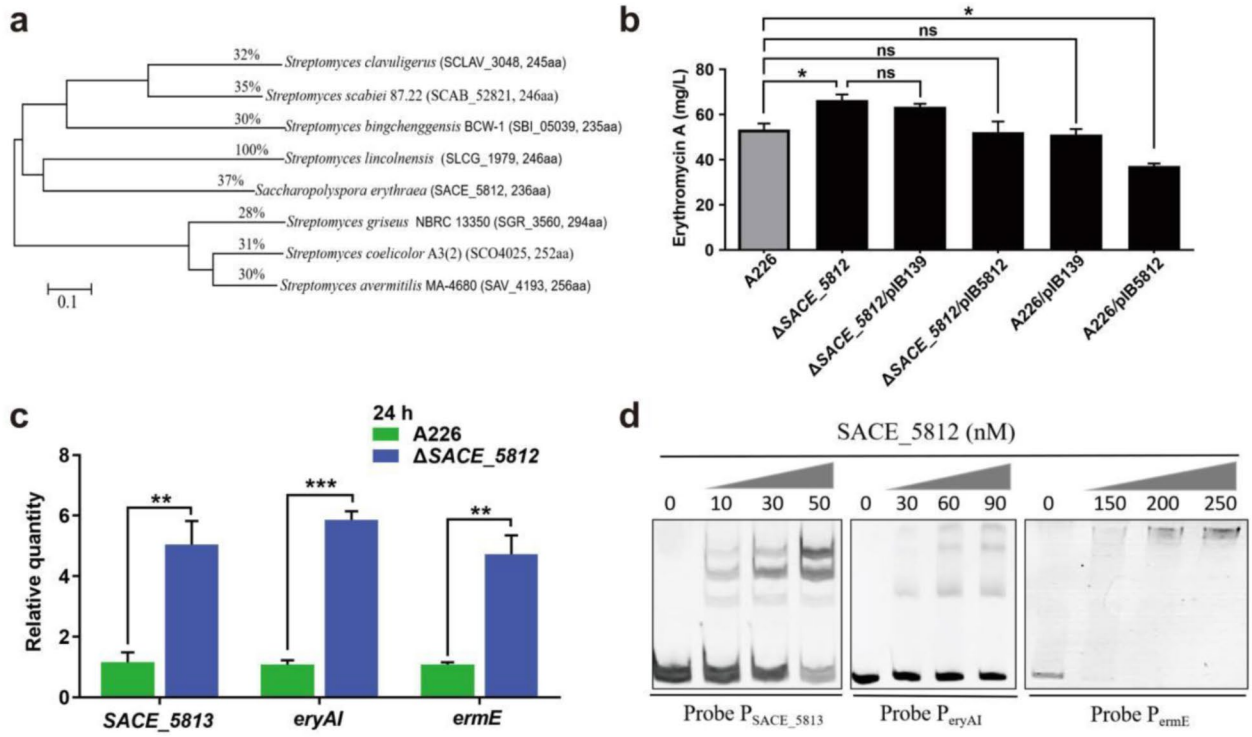

Fig. 7 AbrT homologous protein functions in *Sac. erythraea*. **(a)** Construction of neighbor-joining distance tree depicting AbrT and its homologs. The tree was constructed using the amino acid sequences of AbrT and its homologs in actinomycetes with MEGA. The percentages represent the identities between AbrT and its homologs. **(b)** HPLC analyses of Er-A production in A226 and its derivatives. **(c)** qRT-PCR analyses of *ery* cluster genes and *SLCG_5813* in A226 and Δ*SACE_5812* cultured for 24 h. The mean values of three replicates are shown, with the standard deviation indicated by error bars. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. **(d)** EMSAs of SACE_5812 binding to *SLCG_5813*, *eryAI*, and *ermE* promoter

Abbreviations

Lin	Lincomycin biosynthetic gene
TFs	Transcription factors
TFR	TetR family transcriptional regulator
Lin-A	Lincomycin A
Nt	Nucleotides
HPLC	High-performance liquid chromatography
qRT-PCR	Quantitative real-time PCR
EMSAs	Electrophoretic mobility shift assays
N	Probe $P_{abrT-1980}$
M1 or M2	Single-site mutated DNA fragment
M3	Double-site mutated DNA fragment
MEME	Motif-finding program (MEME)
RFUs	Relative fluorescence units
Er-A	Erythromycin A
LB	Luria Bertani
MM	Minimal medium
YMG	Yeast Malt Glucose
E. coli	Escherichia coli
NTA	Ni^{2+}-nitrilotriacetic acid
OD	Optical density

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12934-024-02498-8.

Supplementary Material 1

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Not applicable.

Author contributions
WH, XY and ZB supervised the experiments. XY, LM and WH conceived and designed research. XY, LM and ZR conducted experiments. LM, PY, WP, ZC and CX analyzed the data. XY and LM wrote the original manuscript. XY and WH edited the manuscript. All authors read and approved the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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