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A novel regulator CdsR negatively regulates cell motility in *Bacillus thuringiensis*

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Cell motility increases the fitness of bacterial cells. Previous research focused on the transcriptional regulator CdsR, which represses cellular autolysis and promotes spore formation in *Bacillus thuringiensis*. However, the targets of CdsR are mostly unknown. Here, we reported a new function of CdsR in regulating cell motility. Mutation of *cdsR* results in increase of cell mobility, and a number of related genes were upregulated compared to wild type HD73. Thus, we investigated the transcription of the *fla/che* gene cluster, which involves in cell mobility and comprises eight operons/genes, including *motAB1*, *cheY-yrhK*, *lamB-cheR*, *yaaR-fliG2*, *cheV-mogR*, *hag1*, *hag2*, and *yjbJ-flgG*. Additionally, the *motAB2* operon was discovered, which consists of homologs genes *motA2* and *motB2* that are like *motA1* and *motB1*. Through promoter-*lacZ* fusion assays and EMSA experiments, it was discovered that CdsR directly regulates the *motAB1*, *cheY-yrhK*, *lamB-cheR*, *yaaR-fliG2*, *cheV-mogR*, *hag1*, *hag2*, *yjbJ-flgG*, and *motAB2* operons by binding to their promoter regions. Importantly, it was confirmed that CdsR is a metalloregulator and the binding to promoter can be inhibited by Cu (II) ions. This research enhances our understanding of the regulation of cell mobility in *B. thuringiensis*.

Keywords *Bacillus thuringiensis*, Transcriptional regulator CdsR, Cell motility, *fla/che* cluster, Cu (II) ions

The bacteria flagellum is a protein complex responsible for bacterial locomotion under various environmental conditions[1]. Bacterial cell motility is defined as the ability of bacteria to move in response to external stimuli, such as gradients of chemokines, growth factors, and extracellular matrix components[2]. Cell chemotaxis, on the other hand, is the directed movement of bacteria towards or away from chemical substances in their environment. It is a type of motility that is driven by the sensing of chemical gradients. Bacterial cell chemotaxis is a tightly coordinated mechanism that enables cells to navigate and respond to their environment in a directed manner[3]. The relationship between cell motility and chemotaxis is crucial for bacterial survival. The bacteria flagellum acts as a propulsion system that allows bacteria to move towards favorable conditions while avoiding harmful environments. The ability is essential for bacterial survival and plays a significant role in various biological processes, including colonization, biofilm formation, and pathogenesis[4].

There are several mechanisms by which the transcriptional regulation of bacterial motility and chemotaxis can occur. These mechanisms involve the control of gene expression through specific transcription factors, signaling pathways, and regulatory elements[5–8]. Specific examples, the Sigma factors initiate transcription of flagellar genes *fla/che* operon in *Bacillus subtilis* and *Escherichia coli*. The *fla/che* operon, which has a long 27 kb *fla/che* operon of 31 genes co-transcribed from the common P*fla/che* promoter in *B. subtilis*[8–10]. DNA-binding proteins, such as the osmoregulation trans-factor OmpR, bind to specific sequences in the *flhDC* operon promoter and repress transcription in *E. coli*[11]. These regulatory mechanisms allow bacteria to respond to environmental cues and adapt their motility and chemotaxis behaviors accordingly.

The ArsR (Arsenical Resistance Operon Repressor) family transcriptional regulators are known for its role in conferring resistance to arsenic acid. This family of transcriptional regulators are found across various prokaryotes and play a crucial role in maintaining intracellular metal ion homeostasis[12,13]. The main function of these ArsR regulators is to act as metal-regulatory proteins that facilitate processes such as metal ion uptake, efflux, sequestration, and detoxification[14]. These actions are particularly important in preserving the equilibrium of metal ions within cells, especially when exposed to harsh environmental conditions. However, studies on the regulation of bacterial cell motility-related gene expression by ArsR family transcriptional regulators are rare.

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B. thuringiensis is a gram-positive, spore-forming bacterium that produces parasporal crystals toxic to a wide range of insect larvae during sporulation[15]. Only a few transcriptional regulators of cell motility have been identified in *B. thuringiensis*. The MogR transcriptional regulator, encoded in the motility locus, was found to repress the expression of motility genes in *B. thuringiensis* 407[16]. PlcR is another important transcriptional regulator of virulence genes, which also affects cell motility[16]. Additionally, the SinI-SinR system, a regulatory system conserved between *B. cereus* and *B. subtilis*, consists of the transcriptional regulator SinR and its anti-repressor SinI. SinR acts as a master transcriptional repressor that controls the switch from a motile to a sessile lifestyle[17,18]. However, the mechanisms of transcriptional regulation for genes related to cell motility in *B. thuringiensis* are not well understood.

Results

Mutation of CdsR results in increased cell motility

Previous research has revealed that the *cdsR* gene in *Bacillus thuringiensis* HD73 (locus tag *HD73_RS03760*), which encodes a novel ArsR family transcriptional regulator, is involved in spore formation and cellular autolysis[19]. To identify putative target genes/operons that are regulated by CdsR, we analyzed the RNA-seq data at T_0 and found that the expression of genes related to cell motility and chemotaxis were up-regulated in the *cdsR* mutant compared to the wild type HD73 (Table 1). To validate these findings from the RNA-seq data, we randomly selected five genes for RT-qPCR analysis. The results showed that the transcription levels of *cheV*, *fliM*, *motA2*, *cheR*, and *fliG1* genes were higher in the *cdsR* mutant than that in HD73 (Fig. 1A), suggesting that the motility and chemotaxis-related genes were up-regulated in the *cdsR* mutant.

To further investigate the impact of *cdsR* deletion on cell motility in *B. thuringiensis* HD73 strains, we conducted a swarming assay. The results showed a significant increase in plaque diameter in Δ*cdsR* (*cdsR* deletion mutant) and a significant decrease in C*cdsR* (complementation strain) compared to HD73 (Fig. 1B). Specifically, the plaque diameter was approximately 3.94 ± 0.39 cm for HD73, whereas the Δ*cdsR* and C*cdsR* displayed a diameter of approximately 8.31 ± 0.43 cm and 1.85 ± 0.14 cm, respectively (Fig. 1C). These findings provide evidence that deletion of the *cdsR* gene leads to a notable increase in cell motility.

Prediction of *che/fla* cluster in *B. thuringiensis* HD73

The *che/fla* gene cluster includes the *che* gene cluster (encapsulating key genes in the chemotaxis pathway) and the *fla* gene cluster (encapsulating key genes in flagellar assembly). Operons in the *che/fla* gene cluster were predicted based on RNA-seq data (accession no. GSE216307) and Rockhopper software[20] (version 2.03), a comprehensive system that supports the various stages of RNA-seq date analysis, including normalizing data from different experiments, quantifying transcript abundance, and testing for differential transcript expression. The *che* gene cluster consists of four operons (*motAB1*, *cheY-yrhK*, *lamB-cheR*, and *cheV-mogR*) (Table 1; Fig. 2A). The *fla* cluster consists of four genes/operons (*yaaR-fliG2*, *hag1*, *hag2*, *yjbJ-flgG*) (Table 1; Fig. 2A). Moreover, *motA* and *motB* genes were responsible for the production of proteins that were involved in bacterial flagellar motility. The *motAB1* operon consists of two genes, *motA1* and *motB1*, which also have homologs in the HD73 genome, *motA2* and *motB2*, respectively (Table 1; Fig. 2A). The *cheY-yrhK* operon consist of five genes (*cheY*, *cheA*, *cheC*, *ygaD*, and *yrhK*). The *lamB-cheR* operon consist of two genes, including *lamB* and *cheR*. The *cheV-mogR* operon consists of two genes, including *cheV* and *mogR*. The *yaaR-fliG2* operon consist of twenty genes (*yaaR*, *flgN*, *flgK*, *flgL*, *fliD*, *fliS*, *yopR*, *flgB*, *flgC*, *fliE*, *fliF*, *fliG1*, *fliH*, *fliI*, *phoU*, *fliK*, *flgD*, *flgE*, *ykuH*, and *fliG2*). The *hag1* and *hag2* were homologous genes of *hag* gene and each was transcribed independently. Finally, the *yjbJ-flgG* operon consist of twelve genes (*yjbJ*, *fliN1*, *fliM*, *fliN2*, *fliN3*, *fliP*, *fliQ*, *fliR*, *flhB*, *flhA*, *flhF*, and *flgG*) (Table 1; Fig. 2A).

CdsR negatively regulates the expression of *fla* gene cluster

The bacteria flagellum is a rotary nanomachine comprised of more than 25 genes that encode various proteins. It comprises three main structural parts: the long helical filament, which acts as a propeller to produce thrust; the basal body, which is a membrane-embedded rotary motor; and the hook, which acts as a universal joint connecting the filament to the basal body[21]. In *B. thuringiensis*, a *fla/che* gene cluster of 45 genes with homology to flagellar-based motility genes is present (Fig. 2B). We studied the transcription and regulation of four operons/genes, which belongs to the *fla* gene cluster, including P*hag1*, P*hag2*, P*yaaR-fliG2* and P*yjbJ-flgG*, respectively (Fig. 2A). The P*hag1-lacZ*, P*hag2-lacZ*, P*yaaR-fliG2-lacZ* and P*yjbJ-flgG-lacZ* fusions were constructed and transformed into HD73 and Δ*cdsR* mutant. The β-galactosidase assay showed that the promoter of P*hag1* had almost no transcriptional activity from T_{-1} to T_6 both in HD73 and Δ*cdsR* (Fig. 2C). Compared with HD73, the transcriptional activity of the P*hag2* increased slightly from T_1 to T_6 in Δ*cdsR*, with no significant difference from T_{-1} to T_0 (Fig. 2E). To determine whether CdsR directly or indirectly regulates the P*hag1* and P*hag2*, we tested the binding of the CdsR protein to the *hag1* and *hag2* promoter regions in an electrophoretic mobility shift assay (EMSA). EMSA result showed that 0.48 nM FAM probe-labeled P*hag1* could bind to CdsR at concentrations of 0.89 μM, 1.34 μM, and 1.79 μM, respectively (Fig. 2D). Notably, a 200-fold excess of unlabeled P*hag1* competed with the labeled P*hag1*, confirming the specific binding (Fig. 2D). Furthermore, when 0.88 nM labeled P*hag2* was exposed to CdsR with concentrations ranging from 0.45 μM to 1.34 μM, the band shift could be detected, indicating that CdsR can bind to the *hag2* promoter region (Fig. 2F). These results strongly supported the theory that CdsR directly regulates the expression of *hag1* and *hag2*.

To validate the transcription of the *yaaR-fliG2* and *yjbJ-flgG* in HD73 and Δ*cdsR*, P*yaaR-fliG2* and P*yjbJ-flgG* promoter fusion with *lacZ* gene were constructed and analyzed by evaluating β-galactosidase activity. The result showed that the transcriptional activity of P*yjbJ-flgG* was increased in Δ*cdsR* compared to that in HD73 (Fig. 2G). EMSA result showed that the probe-labeled P*yjbJ-fliG2* at 0.96 nM binds to CdsR (concentrations from 0.92 μM to 3.66 μM), and competitively inhibited by 200-fold unlabeled promoter, confirming specific

Gene ID	Gene name	ΔcdsR FPKM[a]	HD73 FPKM	Log_2fold-change[b]	Operons	Orientation[c]	Annotations
HD73_RS09605	*motA1*	1934.6	1154.36	1.14	*motAB1*	+	Flagellar motor protein MotA1
HD73_RS09610	*motB1*	105.58	63.42	1.15	*motAB1*	+	Flagellar motor protein MotB1
HD73_RS09615	*cheY*	96.44	56.67	1.17	*cheY-yrhK*	+	Chemotaxis protein CheY
HD73_RS09620	*cheA*	791.5	458.4	1.18	*cheY-yrhK*	+	Chemotaxis protein CheA
HD73_RS09625	*cheC*	152.87	88.55	1.18	*cheY-yrhK*	+	Flagellar motor switch protein CheC
HD73_RS09630	*ygaD*	37.2	21.32	1.21	*cheY-yrhK*	+	Hypothetical protein
HD73_RS09635	*yrhK*	43.27	24.66	1.21	*cheY-yrhK*	+	Hypothetical protein
HD73_RS09640	*cheR*	170.43	96.24	1.23	*lamB-cheR*	-	Chemotaxis methyltransferase CheR
HD73_RS09645	*lamB*	105.02	56.36	1.3	*lamB-cheR*	-	Hypothetical protein
HD73_RS09650	*yaaR*	256.4	135.49	1.32	*yaaR-fliG2*	+	YaaR family protein
HD73_RS09655	*flgN*	193.65	101.12	1.34	*yaaR-fliG2*	+	Flagellar export chaperones FlgN
HD73_RS09660	*flgK*	3458.4	1801.12	1.34	*yaaR-fliG2*	+	Flagellar hook-associated protein FlgK
HD73_RS09665	*flgL*	193.15	99.56	1.35	*yaaR-fliG2*	+	Flagellar hook-associated protein FlgL
HD73_RS09670	*fliD*	119.18	61.13	1.36	*yaaR-fliG2*	+	Flagellar hook-associated protein 2
HD73_RS09675	*fliS*	142.13	72.26	1.38	*yaaR-fliG2*	+	Flagellar secretion chaperone FliS
HD73_RS09680	*yopR*	129.56	65.5	1.39	*yaaR-fliG2*	+	Hypothetical protein
HD73_RS09685	*flgB*	822.32	409.15	1.4	*yaaR-fliG2*	+	Flagellar basal-body rod protein FlgB
HD73_RS09690	*flgC*	320.46	158.72	1.42	*yaaR-fliG2*	+	Flagellar basal-body rod protein FlgC
HD73_RS09700	*fliF*	610.15	296.79	1.44	*yaaR-fliG2*	+	Flagellar M-ring protein FliF
HD73_RS09705	*fliG1*	124.99	53.36	1.63	*yaaR-fliG2*	+	Flagellar motor switch protein FliG1
HD73_RS09710	*fliH*	154.92	65.18	1.65	*yaaR-fliG2*	+	Flagellar assembly protein FliH
HD73_RS09715	*fliI*	408.35	168.23	1.68	*yaaR-fliG2*	+	Flagellar protein export ATPase FliI
HD73_RS09720	*phoU*	7923.49	3101.4	1.75	*yaaR-fliG2*	+	Cytoplasmic protein
HD73_RS09725	*fliK*	155.23	60.39	1.77	*yaaR-fliG2*	+	Flagellar hook-length control protein FliK
HD73_RS09730	*flgD*	245.96	84.9	1.94	*yaaR-fliG2*	+	Flagellar hook assembly protein FlgD
HD73_RS09735	*flgE*	329.04	113.26	1.94	*yaaR-fliG2*	+	Flagellar hook protein FlgE
HD73_RS09740	*ykuH*	179.5	61.9	1.94	*yaaR-fliG2*	+	Hypothetical protein
HD73_RS09745	*fliG2*	1109.88	363.8	2.01	*yaaR-fliG2*	+	Flagellar motor switch protein FliG
HD73_RS09750	*cheV*	774.38	252.27	2.02	*cheV-mogR*	+	Chemotaxis protein CheV
HD73_RS09770	*yjbJ*	141.81	45.29	2.04	*yjbJ-flgG*	+	Lysozyme-like protein
HD73_RS09775	*fliN1*	424.1	133.85	2.07	*yjbJ-flgG*	+	Flagellar motor switch protein FliN1
HD73_RS09780	*fliM*	293.42	75.49	2.36	*yjbJ-flgG*	+	Flagellar motor switch protein FliM
HD73_RS09785	*fliN2*	83.28	54.55	1.01	*yjbJ-flgG*	+	Flagellar motor switch protein FliN2
HD73_RS09790	*fliN3*	244.34	160.12	1.02	*yjbJ-flgG*	+	Flagellar motor switch protein FliN3
HD73_RS09795	*fliP*	672.6	436.78	1.02	*yjbJ-flgG*	+	Flagellar biosynthesis protein FliP
HD73_RS09800	*fliQ*	68.19	44.46	1.02	*yjbJ-flgG*	+	Flagellar biosynthesis protein FliQ
HD73_RS09805	*fliR*	161.98	105.12	1.04	*yjbJ-flgG*	+	Flagellar biosynthesis protein FliR
HD73_RS09820	*flhF*	334.11	213.68	1.04	*yjbJ-flgG*	+	Flagellar biosynthesis protein FlhF
HD73_RS09825	*flgG*	447.8	284.92	1.05	*yjbJ-flgG*	+	Flagellar basal-body rod protein FlgG
HD73_RS23925	*motB2*	653.98	354.54	1.27	*motAB2*	+	Flagellar motor protein MotB2
HD73_RS23930	*motA2*	1404.14	761.50	1.28	*motAB2*	+	Flagellar motor stator protein MotA2

Table 1. Cell motility of upregulated genes expressed in the Δ*cdsR* mutant. [a]The differentially expressed genes associated with cell motility were analyzed according to fragments per kilobase of transcript per million mapped reads (FPKM). [b]Differentially expressed genes were screened based on a log_2fold-change ≥ 1 and a false discovery rate cutoff of $P \leq 0.05$, using RNA-Seq data. [c]The plus (+) and minus (-) signs represent the forward and reverse strands of the DNA sequence, respectively.

binding (Fig. 2H). The result suggests that CdsR directly binds to the *yjbJ-flgG* promoter region. In addition, the β-galactosidase activity showed that the P*yaaR-fliG2* promoter transcriptional activity increased from T_{-1} to T_6 in Δ*cdsR* compared to HD73 (Fig. 2I), and EMSA confirmed that CdsR directly binds to the P*yaaR-fliG2* promoter (Fig. 2J). These results collectively suggest that CdsR negatively regulates the expression of *hag1*, *hag2*, *yjbJ-flgG*, and *yaaR-fliG2* operons/genes.

CdsR negatively regulates the expression of *che* gene cluster

We then studied the transcription and regulation of *che* gene cluster, including *cheY-yrhK*, *lamb-cheR*, and *cheV-mogR*, respectively (Fig. 3A). The signaling core of the chemotaxis pathway consists of chemoreceptors (MCPs),

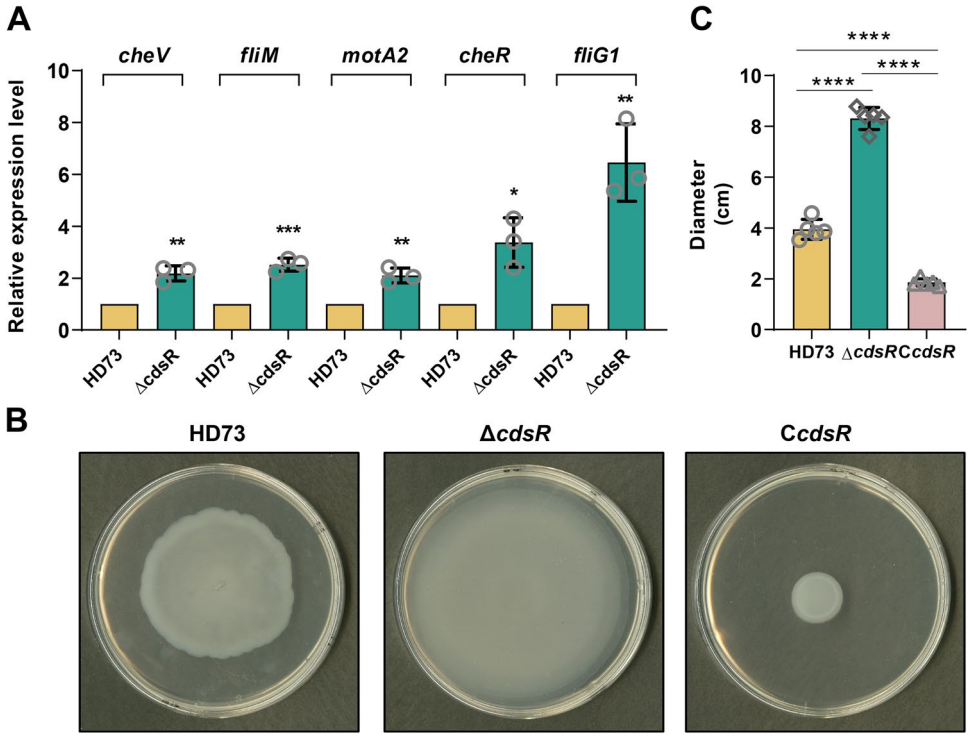

Figure 1. Comparison of cell motility of the *B. thuringiensis* HD73 and Δ*cdsR*. (**A**) Analysis of *cheV*, *fliM*, *motA2*, *cheR*, and *fliG1* expression in Δ*cdsR* and HD73 strains at T_0 by RT-qPCR. Experiments were conducted in triplicate. Error bars represent standard deviations, and data were analyzed using a t-test (*, $P < 0.05$. **, $P < 0.01$. ***, $P < 0.001$.). (**B**) Cell motility of the Δ*cdsR* (mutant strain) and C*cdsR* (complementary strain) were compared with that of the HD73 (wild-type strain). The cell motility of *B. thuringiensis* strains was observed in LB solid medium containing 0.5% agar. (**C**) Comparison of plaque diameters of the Δ*cdsR*, C*cdsR*, and HD73. Five independent experiments were used to analyze the data by t-test (****, $P < 0.0001$).

a histidine kinase CheA, and a methylated receptor CheR that controls the autophosphorylation activity of CheA[22]. CheA then transfers the phosphoryl group to the CheY, a response regulator. CheV is a scaffold protein that interacts with both CheA and CheY, regulating their activity. The phosphorylated form of CheY (CheY-P) induces clockwise rotation of the flagellar motor, promoting transient tumbles that reorient the cell body (Fig. 3B). To detect the transcription dynamics of these operons, the *cheY-yrhK* (P*cheY-yrhK*), *lamB-cheR* (P*lamB-cheR*), and *cheV-mogR* (P*cheV-mogR*) promoters were fused to *lacZ* reporter gene. We identified the expression of these three operons in both HD73 and Δ*cdsR* through β-galactosidase assay. The result showed that the activities of the P*cheY-yrhK* and P*cheV-mogR* were significantly higher in Δ*cdsR* than in HD73 from T_{-1} to T_6 (Fig. 3C and D). P*lamB-cheR* activity was similar in Δ*cdsR* and HD73 from T_{-1} to T_3, but higher in Δ*cdsR* from T_4 to T_6 (Fig. 3E). These results clearly indicate that CdsR represses the transcription of *cheY-yrhK*, *lamB-cheR*, and *cheV-mogR*.

To determine whether CdsR binds to the promoter regions of *cheY-yrhK*, *lamB-cheR*, and *cheV-mogR*, EMSA was performed. Various concentrations of the CdsR protein were used to bind to the 1.18 nM probe-labeled P*cheY-yrhK*. The result showed that high concentration (1.60 μM and 2.13 μM) of CdsR can bind to the labeled P*cheY-yrhK*, and this binding is competed by 200-fold excess of unlabeled promoter (Fig. 3F). Similarly, FAM-labeled *cheV-mogR* promoter fragments were incubated with different amounts of CdsR and assayed for the formation of Protein-DNA complexes. Slower-migrating probe-protein complexes were observed upon incubation with increasing amounts of CdsR (Fig. 3G). CdsR protein with a concentration of 3.20 μM and 4.26 μM could also bind to probe-labeled P*lamB-cheR*, and competitively inhibited by 200-fold unlabeled promoter, confirming specific binding (Fig. 3H). These results collectively suggest that CdsR negatively regulates the expression of *cheY-yrhK*, *lamB-cheR*, and *cheV-mogR*.

Transcriptional activity of MotAB flagellar motor genes

To confirm whether CdsR regulates the *motAB1* and *motAB2* operons, β-galactosidase activities were performed in HD73 and Δ*cdsR*, respectively. The β-galactosidase assay showed that in HD73, the activity of the P*motAB1* remained low from T_{-1} to T_6. However, in Δ*cdsR*, the transcriptional activity of the P*motAB1* was significantly higher than in HD73 from T_{-1} to T_6 and increased significantly from T_0 to T_6 (Fig. 4A). The result suggests that *motAB1* expression is suppressed by CdsR. To confirm whether the CdsR protein could bind to *motAB1* promoter region, we conducted an EMSA which showed that high concentration (1.71 μM) of CdsR is able to bind to 1.18 nM of probe-labeled P*motAB1*. Notably, 200-fold excess of unlabeled P*motAB1* could compete

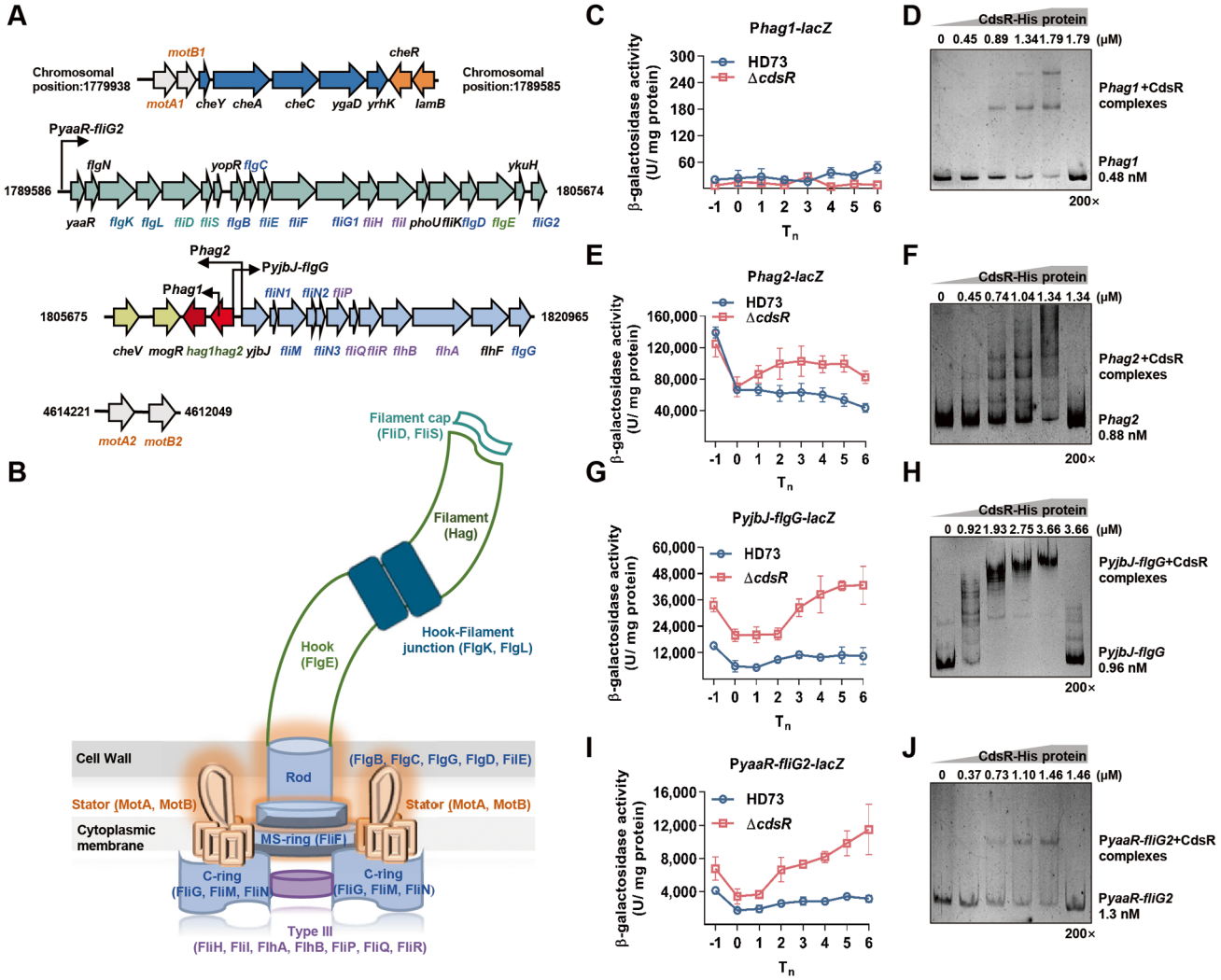

Figure 2. Transcription of *hag1*, *hag2*, *yjbJ-flgG*, and *yaaR-fliG2* operons/genes are regulated by CdsR. (**A**) RNA-seq data were analysed using Rockhopper software (version 2.03) and operons in the *che*/*fla* gene cluster were predicted in *B. thuringiensis*. Genes of the same color in the figure belong to a single operon. Open reading frames (ORFs) are represented by broad arrows, and promoter regions are represented by black arrows. The different operons in the figure are described in Table 1. The genetic organization of the *fla* gene cluster, including the *yaaR-fliG2*, *hag1*, *hag2*, and *yjbJ-flgG* operons, are represented by green, dark red, light red, and blue arrows, respectively. The numbers at the ends of the lines represent the locations in the genome. (**B**) Schematic representation of the *B. thuringiensis* flagellum. The flagellum is divided into three structural parts: the helical filament, which acts as a propeller; the basal body, which is embedded in the cytoplasmic membrane; and the hook, which connects the filament to the basal body. The basal body acts as a bidirectional rotary motor consisting of a rotor ring complex and several stator units around the rotor. (**C**) β-galactosidase activity of P*hag1-lacZ* in the *cdsR* mutant strains and HD73 wild-type strain. T_0 is the end of exponential phase, and T_n is n hours after T_0. Each value represents the mean of at least three replicates. (**D**) EMSA experiment confirms CdsR binding to *hag1* gene promoter fragment (317 bp). The last lane, incubation of 200-fold greater unlabeled P*hag1* probe mixed with the labeled P*hag1* probe and 1.79 μM CdsR. (**E**) β-galactosidase activity of P*hag2-lacZ* in Δ*cdsR* and HD73. (**F**) EMSA experiment confirms CdsR binding to *hag2* gene promoter fragment (610 bp). (**G**) β-galactosidase activity of P*yjbJ-flgG-lacZ* in Δ*cdsR* and HD73. (**H**) EMSA experiment confirms CdsR binding to *yjbJ-flgG* operon promoter fragment (316 bp). (**I**) β-galactosidase activity of P*yaaR-fliG2-lacZ* in Δ*cdsR* and HD73. (**J**) EMSA experiment confirms CdsR binding to *yaaR-fliG2* operon promoter fragment (235 bp).

with the labeled promoter, confirming a specific binding (Fig. 4B). These results indicate that *motAB1* operon is negatively regulated by CdsR.

Furthermore, to investigate whether *motAB2*, a redundant operon of *motAB1*, is also regulated by CdsR, β-galactosidase assay and EMSA were performed. The *motAB2* operon promoter was fused with the *lacZ* reporter gene and transformed into HD73 and Δ*cdsR*. β-galactosidase assay was measured, which showed that the activity of P*motAB2* increased from T_{-1} to T_6 and reached the highest level at T_6 in Δ*cdsR*. However, the

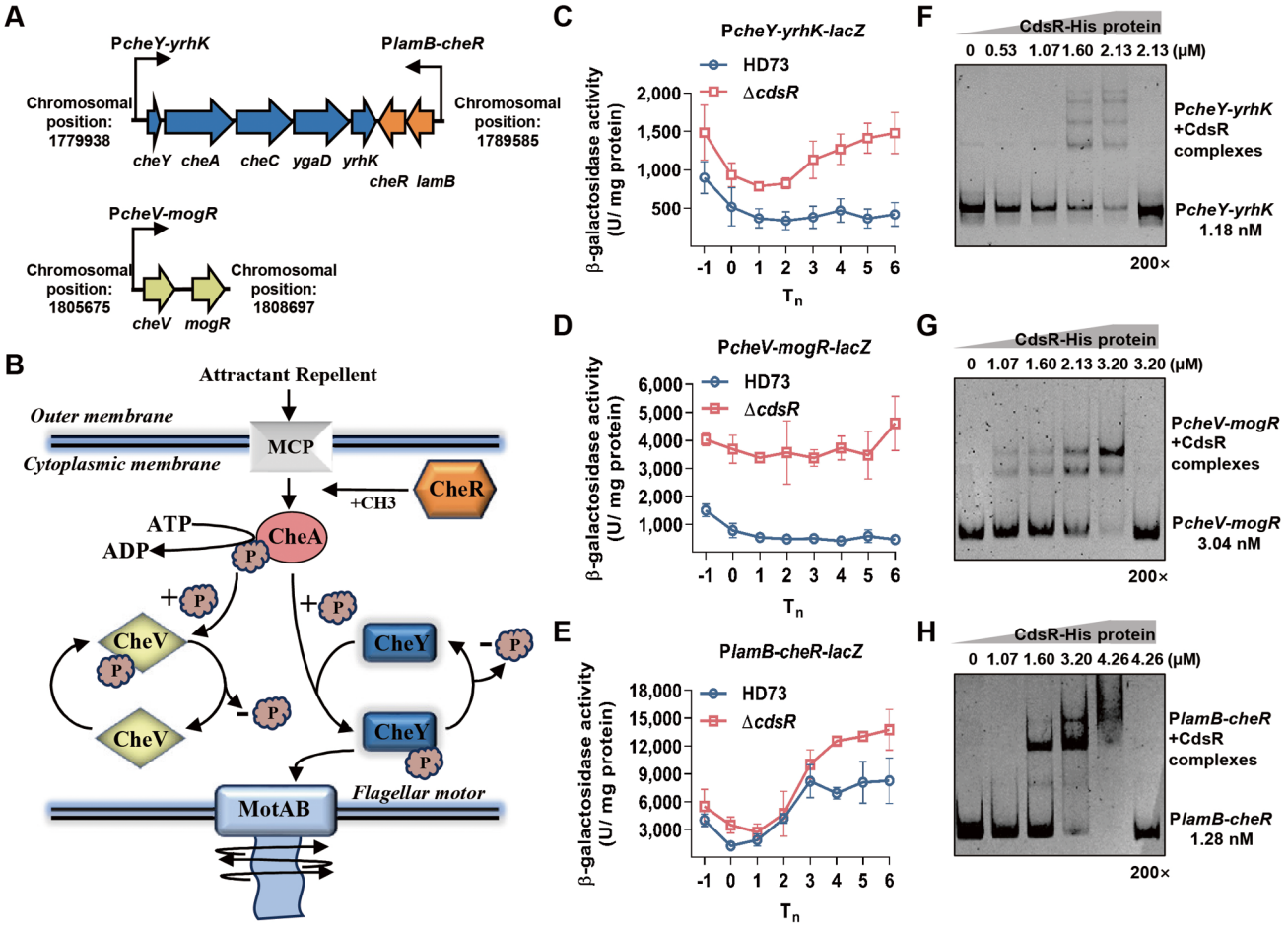

Figure 3. Transcription of *cheY-yrhK, lamb-cheR*, and *cheV-mogR* operons/genes are regulated by CdsR. (**A**) Genetic organization of the *che* gene cluster. ORFs are represented by different color arrows, and promoter regions are represented by black arrows. Blue, orange, and light-yellow arrows indicate *cheY-yrhK*, *lamB-cheR*, and *cheV-mogR* operon, respectively. The numbers at the ends of the lines represent the locations in the genome for the two end bases. (**B**) Chemotaxis signaling pathway of *B. thuringiensis*. The P represents the phosphate group in the figure. (**C**) β-galactosidase activity of P*cheY-yrhK-lacZ* in the *cdsR* mutant strains and HD73 wild-type strain. Each value represents the mean of at least three replicates. (**D**) β-galactosidase activity of P*cheV-mogR-lacZ* in Δ*cdsR* and HD73. (**E**) β-galactosidase activity of P*lamB-cheR-lacZ* in Δ*cdsR* and HD73. (**F**) EMSA experiment confirms CdsR binding to *cheY-yrhK* operon promoter fragment (260 bp). The last lane, incubation of 200-fold greater unlabeled P*cheY-yrhK* probe mixed with the labeled P*cheY-yrhK* probe and 2.13 μM CdsR. (**G**) EMSA experiment confirms CdsR binding to *cheV-mogR* operon promoter fragment (203 bp). (**H**) EMSA experiment confirms CdsR binding to *lamB-cheR* operon promoter fragment (239 bp).

transcriptional activity of P*motAB2* was inhibited from T_{-1} to T_6 in HD73 (Fig. 4C). Then, we tested the binding of the CdsR protein to the *motAB1* operon promoter region. EMSA result showed that various concentrations of the CdsR protein were used to binding 1.14 nM of the P*motAB2*-labeled probe. At a concentration of 1.28 μM, the CdsR protein was unable to effectively bind to the P*motAB2*-labeled probe. However, at a higher concentration (3.83 μM), CdsR was able to completely shift the probe-labeled P*motAB2*. Notably, a 200-fold excess of unlabeled P*motAB2* competed with the labeled P*motAB*, confirming the specific binding (Fig. 4D). These results collectively indicate that *motAB2* operon is negatively regulated by CdsR.

CdsR is a metalloregulatory protein

ArsR family transcription factor is a winged-helix-turn-helix transcriptional regulator that may function as a metal-sensing protein, modulating the expression of genes associated with metal homeostasis[14]. CdsR consists of 95 amino acids and belongs to the ArsR family of transcriptional regulators, which include a single helix-turn-helix (HTH) DNA-binding domain ranging from 15 to 90 amino acid residues[19]. To identify which metal ions, regulate CdsR activity, we next examined the effect of metal ions on the binding of CdsR protein to the *cheV-mogR* operon promoter. EMSA experiment was conducted under metal ions condition (Fig. 5A, lanes 1–3). When Cu (II) was co-incubated with the probe-labeled probe promoter fragment and 1.22 μM CdsR, the protein-DNA complex formation was disrupted (Fig. 5A, lane 4). However, this phenomenon was not observed with the addition of Ca (II), Mg (II), Cd (II), Ni (II), Mn (II), and Zn (II) (Fig. 5A, lanes 5–10). The results

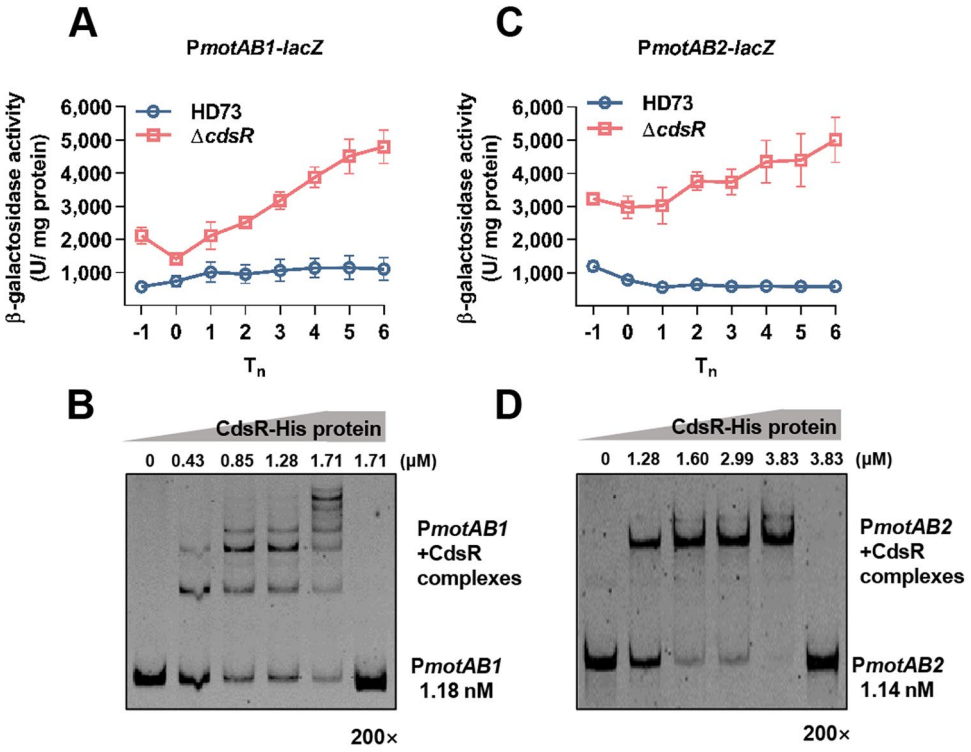

Figure 4. Transcription of*motAB1* and*motAB2* operons depended on CdsR. (**A**) β-galactosidase activity of P*motAB1-lacZ* in Δ*cdsR* and HD73 in SSM medium at T_{-1} to T_6. (**B**) EMSA experiment confirms CdsR binding to *motAB1* gene promoter fragment (260 bp). The last lane, incubation of 200-fold greater unlabeled P*motAB1* probe mixed with the labeled P*motAB1* probe and 1.71 μM CdsR. (**C**) β-galactosidase activity of P*motAB2-lacZ* in Δ*cdsR* and HD73. (**D**) EMSA experiment confirms CdsR binding to *motAB2* promoter gene region (269 bp).

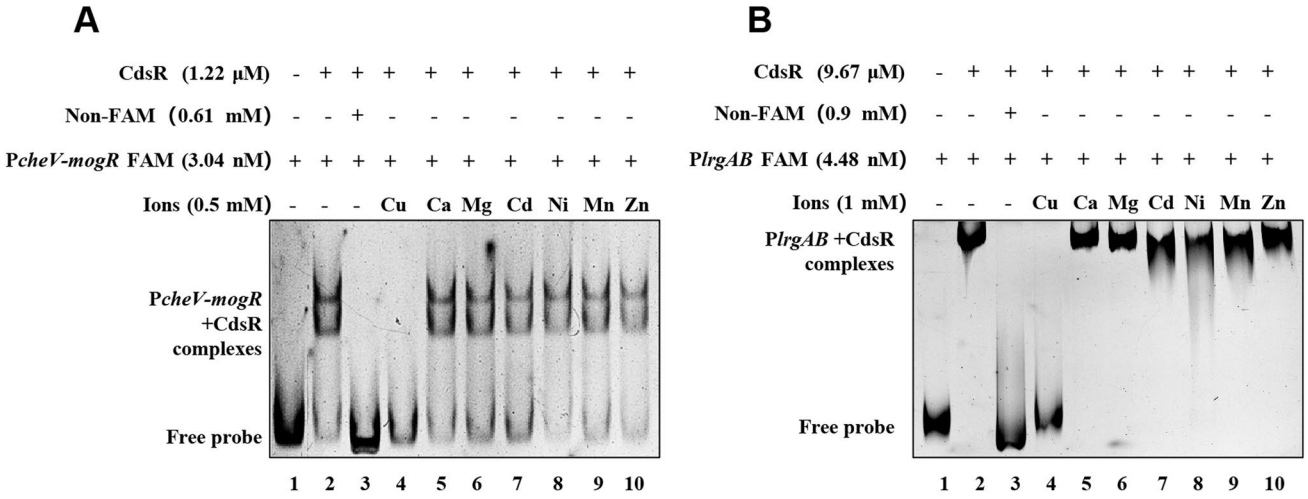

Figure 5. The metal Cu (II) ions inhibit CdsR binding to promoters. (**A**) EMSA experiment confirms that the binding of CdsR to P*cheV-mogR* is specifically inhibited by Cu (II) ions. The probe-labeled *cheV-mogR* operon promoter was incubated with different kinds of metal ions (0.5 mM) with 1.22 μM concentrations of CdsR protein or without CdsR protein. (**B**) EMSA experiment confirms that the binding of CdsR to P*lrgAB* is specifically inhibited by Cu (II) ions. The probe-labeled *lrgAB* operon promoter was incubated with different kinds of metal ions (1 mM) with 9.67 μM concentrations of CdsR protein or without CdsR protein.

suggest that Cu (II) specifically impairs the DNA-binding ability of CdsR, indicating that CdsR may be a Cu (II)-binding protein.

Additionally, previous studies have shown that CdsR has a negative regulatory effect on the *lrgAB* gene, which encodes holin-like protein[19]. To confirm whether the addition of Cu (II) disrupts the binding of CdsR to the *lrgAB* promoter, we conducted EMSA experiments to reconfirm that the CdsR protein responds to Cu (II). EMSA was performed with the CdsR protein directly binding to the P*lrgAB* promoter as a positive control (Fig. 5B, lanes 1–3). When Cu (II) was co-incubated with the labelled P*lrgAB* promoter fragment and 9.67 μM concentrations of CdsR, CdsR protein-P*lrgAB* promoter complex formation was disrupted (Fig. 5B, lane 4). However, no disruption of protein-DNA complex formation was observed when adding Ca (II), Mg (II), Cd (II), Ni (II), Mn (II), and Zn (II) (Fig. 5B, lanes 5–10). This result again suggests that Cu (II) specifically inhibits the function of CdsR. In turn, CdsR may regulate the expression of certain genes and maintain homeostasis by responding to the intracellular concentration of Cu (II) ions.

To further investigate the effect of the presence and absence of Cu(II) on cell motility in *B. thuringiensis* HD73 strains, we also conducted a swarming assay. The results showed that the plaque diameter of HD73 strain with Cu(II) was significantly increased in the presence of Cu(II) compared to the absence of Cu(II) (Fig. 6A). Specifically, the plaque diameter of HD73 in the absence of Cu(II) was approximately 3.50 ± 0.26 cm, whereas the plaque diameter of HD73 in the presence of Cu(II) was approximately 5.57 ± 0.15 cm (Fig. 6B). These results indicate that the presence of Cu(II) significantly increases the cell motility of the HD73 wild strain.

Discussion

B. subtilis is a Gram-positive bacterium that possesses multiple flagella on its cell surface, like *E. coli* and *Salmonella*. However, the structure and function of the flagella in *B. subtilis* differ from those in *E. coli* and *Salmonella*[21]. For example, thirty-two genes required for basal body synthesis are concentrated in the large 27 kb *fla/che* operon, which is expressed by RNA polymerase and the vegetative sigma factor σ^A. Once hook assembly is complete, the alternative sigma factor σ^D is activated to express a set of genes dedicated to filament assembly and rotation[5]. In contrast, the *B. thuringiensis fla/che* gene cluster on cell motility consists of 45 genes (Fig. 2A), and no orthologs of σ^D have been identified in the *B. thuringiensis* genome. In this study, we identified for the first time that the ArsR family transcriptional regulator CdsR directly regulates the *fla/che* gene cluster expression in *B. thuringiensis*. More importantly, we predicted eight operons in the *fla/che* gene cluster (Fig. 2A), and there may be other operons that we will follow up with RT-PCR (Reverse Transcription PCR) for detailed and accurate validation.

Previous studies have found that CdsR is highly conserved in the *B. cereus* group[19]. Therefore, it is possible that the ArsR family transcriptional regulator CdsR directly regulates expression of the *fla/che* gene cluster in the *B. cereus* group. At least seven species make up the group, including *B. cereus*, *B. anthracis*, *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*, *B. pseudomycoides*, and *B. cytotoxicus*. These species can be found in various environments, such as soil, air, and water[23]. Most strains of *B. cereus*, *B. thuringiensis*, *B. weihenstephanensis*, and *B. cytotoxicus* are motile due to peritrichous flagella, while *B. anthracis*, *B. mycoides*, and *B. pseudomycoides* are non-motile[23–25]. Since CdsR is highly conserved among the four strains in *B. cereus* (100% amino acid similarity), *B. thuringiensis* (100%), and *B. cytotoxicus* (95%). It is hypothesized that CdsR may regulate cell motility. The study of the regulatory mechanisms of cell motility in all other bacteria of the *B. cereus* group will be instructive.

The ArsR family transcriptional regulator is widely distributed among microorganisms and is involved in diverse cellular events[26,27]. Its primary function is to act as a metal sensor, helping to maintain intracellular homeostasis[12,28]. The role of ArsR family transcriptional regulator is to repress the genes or operons expression when related to stress-inducing concentrations of various heavy metal ions[27]. Several studies have shown that the presence of metal ions inhibits DNA-binding activity of ArsR family members, such as ArsR-SmtB, MerR, CsoR-RcnR, CopY, DtxR, Fur, NikR, CmtR, and NmtR transcriptional regulators[13,26]. In *Brucella*, ArsR6 autoregulates its own expression to maintain bacterial intracellular Cu (II) and Ni (II) homeostasis, which favors bacterial

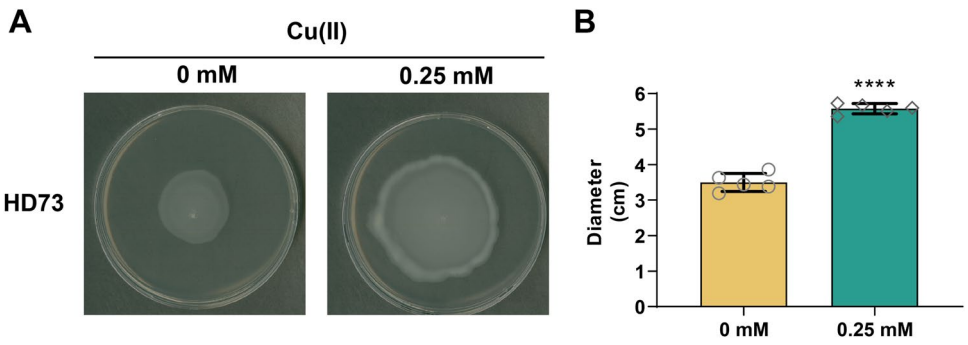

Figure 6. Comparison of cell motility of HD73 strains in the presence and absence of Cu(II). (**A**) The cell motility of HD73 strain was compared in the presence and absence of Cu(II). The cell motility of *B. thuringiensis* strains was observed in LB solid medium containing 0.5% agar. (**B**) Comparison of plaque diameters of the HD73 strain in the presence and absence of Cu(II). Five independent experiments were used to analyze the data by t-test (****, $P < 0.0001$).

survival in hostile environments[29]. In this study, we found for the first time that the transcriptional regulator CdsR can respond to Cu (II) in vitro (Fig. 5). Subsequently, we will focus on the key sites in the CdsR amino acid sequence that bind Cu (II) and how CdsR senses changes in the concentration of intracellular Cu (II) to precisely regulate target genes. Despite the presence of Cu (II) disrupts the binding of CdsR to target genes, however, we did not address in vitro experiments of CdsR binding to Cu (II). Additionally, it is unclear whether CdsR binds Cu inside the cell and how it senses changes in the concentration of Cu inside the bacteria cell. These questions will be addressed in subsequent studies.

The *fla/che* gene cluster has eight operons/genes, including *motAB1*, *cheY-yrhK*, *lamb-cheR*, *yaaR-fliG2*, *cheV-mogR*, *hag1*, *hag2*, *yjbJ-flgG*. In addition, a *motAB2* operon were investigated. The expression of *motAB1*, *cheY-yrhK*, *lamb-cheR*, *yaaR-fliG2*, *cheV-mogR*, *hag1*, *hag2*, *yjbJ-flgG* and *motAB2*, which are controlled by CdsR, are negatively regulated. Furthermore, when higher concentrations of Cu (II) are present in *B. thuringiensis* cell, CdsR may bind Cu (II), which in turn prevents CdsR from binding to the P*motAB1*, P*cheY-yrhK*, P*lamb-cheR*, P*yaaR-fliG2*, P*cheV-mogR*, P*hag1*, P*hag2*, P*yjbJ-flgG* and P*motAB2* promoter regions (Figs. 2, 3 and 4). In conclusion, the *fla/che* gene cluster has different expression and regulation in different bacteria. The data presented in this study may deepen our understanding of the transcriptional mode of the *fla/che* gene cluster, and thus help us to better understand the regulation mechanism of bacterial motility-associated gene cluster.

A previous study showed that CdsR is a negatively autoregulated transcriptional regulator. CdsR consists of 95 amino acids and belongs to the ArsR family of transcriptional regulators, which include a single helix-turn-helix (HTH) DNA-binding domain ranging from 15 to 90 amino acid residues. Furthermore, the genes upstream and downstream of *cdsR* include *istA*, *sspH*, and *dtpA*, which encode the IS21-like element IS232 family transposase, acid-soluble spore protein H, and peptide MFS transporter, respectively[19]. To obtain a deeper understanding of the regulator motifs of the ArsR family transcriptional regulator CdsR, we analyzed a conserved sequence of target genes/operons (such as *hag1*, *hag2*, *yjbJ-flgG*, *yaaR-fliG2*, *cheY-yrhK*, *cheV-mogR*, *lamb-cheR*, *motAB1*, *motAB2*, *lrgAB*, *cdsR*, and *cwlD*) directly regulated by CdsR using the MEME website (https://meme-suite.org/meme/tools/meme). The results showed that the alignment of the 12 promoter regions confirmed the presence of a CdsR binding site rich in G bases. The 12 promoters' sequences were aligned and the conserved sequence WGAGGKGGYTC was identified for CdsR regulated genes (Table S3 and Fig. 7). In a follow-up study, we are going to confirm whether the CdsR binding site in the promoter regions of the 12 target genes is this predicted site by a DNase I footprinting protection assay.

In this study, we focused on the transcriptional regulation of the *fla/che* gene cluster, which is involved in cell motility and chemotaxis in *B. thuringiensis* HD73. The RNA-seq data of the *cdsR* deletion mutant was investigated, and it was found that the expression of genes associated with cell motility were all up-regulated in the *cdsR* deletion mutant compared to the HD73 wild-type. Additionally, deletion of *cdsR* resulted in enhanced cell motility. Subsequently, *motAB1*, *cheY-yrhK*, *lamB-cheR*, *cheV-mogR*, *yaaR-fliG2*, *hag1*, *hag2*, and *yjbJ-flgG* operons/genes were found at the *fla/che* gene cluster in *B. thuringiensis* HD73. The results of the β-galactosidase activity and Electrophoretic mobility shift assay (EMSA) suggest that CdsR regulates the expression of *motAB1*, *motAB2*, *cheY-yrhK*, *lamB-cheR*, *cheV-mogR*, *yaaR-fliG2*, *hag1*, *hag2*, and *yjbJ-flgG*. Furthermore, we found that the ArsR family transcriptional regulator CdsR is inhibited by metallic Cu (II) ions. This work provides new insights into the regulation of cell motility in *B. thuringiensis*.

Materials and methods

Bacterial strains, plasmids, and growth conditions

Bacillus thuringiensis subsp. *kurstaki* HD73 (hereafter designated as HD73, reference genome sequence: NC_020238.1) and derivatives were grown at 30 °C in Luria–Bertani (LB) medium (1% tryptone, 0.5% yeast extract, and 0.5% NaCl) or on solid LB medium supplemented with 1.5% agar. Schaeffer's sporulation medium[30] (SSM; 0.8% nutrient broth, 0.012% $MgSO_4$, 0.1% KCl, 0.5 mM NaOH, 1 mM $Ca(NO_3)_2$, 0.01 μM $MnCl_2$, and 1

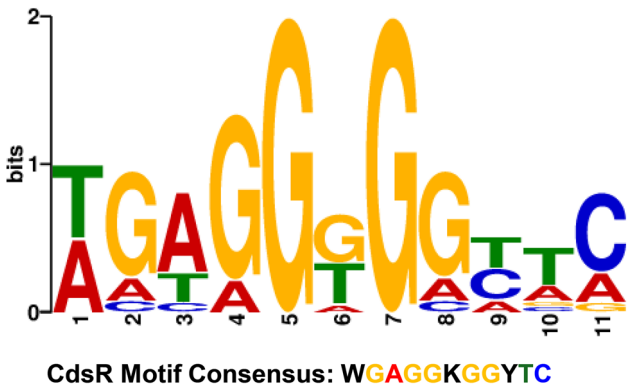

Figure 7. Characterization of a CdsR conserved DNA sequence. The motif of the CdsR-binding DNA was predicted by MEME. The colored region corresponds to a 11-bp DNA sequence highly conserved in the 12 promoter regions. The height of each column indicates the sequence conservation at that position, while the height of symbols within the stack indicates the relative frequency of each nucleic acid at that position. Motif consensus of CdsR binding promoter regions is presented. W is A or T; K is G or T; Y is C or T.

μM $FeSO_4$) was used to observe the development of bacterial cells. *Escherichia coli* TG1 was used for molecular cloning experiments, and *E. coli* ET was used for producing non-methylated plasmid DNA for *B. thuringiensis* transformations[31,32]; both strains were grown at 37 °C in LB medium. The antibiotics were added at the following concentrations for the growth of *B. thuringiensis*: 5 μg/ml erythromycin and 50 μg/ml kanamycin. For the growth of *E. coli*, 100 μg/ml of ampicillin was added when needed. The bacterial strains and plasmids used in this study are summarized in Table S1 in the Supplementary Materials.

DNA manipulation and transformation

Plasmid DNA was extracted from *E. coli* cells with a Plasmid Miniprep Kit (Axygen, Beijing, China). Restriction enzymes and T4 DNA ligase (Takara Biotechnology Corporation, Dalian, China) were used according to the manufacturer's instructions. PCR was performed with the high-fidelity PrimeStar HS DNA polymerase (Takara Biotechnology Corporation, Beijing, China) or *Taq* DNA polymerase (BioMed, Beijing, China). DNA fragments were purified from 1% agarose gels, using the AxyPrep DNA Gel Extraction Kit (Axygen, Beijing, China). Standard transformation procedures for *E. coli*[33], and *B. thuringiensis*[34] were described previously.

Reverse transcription (RT)-qPCR assay

The total RNA was extracted from HD73 and Δ*cdsR* cells collected at T_0 (T_0 indicates the end of the exponential growth phase) using the RNAprep Pure Kit (Aidlab, Beijing, China) according to the manufacturer's instructions. The extracted RNA was then subjected to reverse transcription using the HiScript II Q RT SuperMix (Vazyme, Nanjing, China) to synthesize cDNA. The cDNA samples were diluted to a concentration of 200 ng/μL for subsequent analysis. The transcriptional levels of the target genes were determined by conducting RT-qPCR using the ChamQ Universal SYBR qPCR master mix (Vazyme, Nanjing, China). The 16 S rRNA gene was utilized as an internal control to normalize the expression of the target genes. The specific primer sequences for RT-qPCR analysis are listed in Table S2 in the Supplementary Materials. Finally, the RT-qPCR data analysis was performed using the $2-\Delta\Delta Ct$ method. Subsequently, the data are expressed as the mean fold change relative to the control samples, which were used to assess the relative expression levels of the target genes in HD73 and Δ*cdsR* cells. Significant differences were determined by Student's t-test (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; and ****, $P<0.0001$). Statistical analysis was performed on GraphPad Prism 9 software.

Swarming assay

HD73 and Δ*cdsR* strains were grown in LB medium to an OD_{600} of 1.0. For the swarming assay, 1 μL of culture was spotted onto a LB (0.5% agar) and incubated at 30 °C for 12 h. Photographs were obtained using the ImageScanner III (Cytiva, Stockholm, Sweden).

β-galactosidase activity assays

The *motAB1* (P*motAB1*, 260 bp) and *motAB2* (P*motAB2*, 269 bp) gene promoters were amplified from HD73 genomic DNA using the primers P*motAB1*-F/P*motAB1*-R and P*motAB2*-F/P*motAB2*-R, respectively. The primers used in this study are listed in Table S2 in the Supplementary Materials. The P*motAB1* and P*motAB2* were digested with PstI and BamHI, followed by ligation into the linearized pHT304-18Z plasmid, which harbors a promoter-less *lacZ*[35], to obtain the recombinant plasmids 304P*motAB1* and 304P*motAB2*. The 304P*motAB1* and 304P*motAB2* plasmid were introduced into HD73 and Δ*cdsR*, resulting in HD(P*motAB1*), Δ*cdsR*(P*motAB1*), HD(P*motAB2*) and Δ*cdsR*(P*motAB2*), respectively. The HD(P*motAB1*), Δ*cdsR*(P*motAB1*), HD(P*motAB2*) and Δ*cdsR*(P*motAB2*) strains were validated by PCR.

The promoters' sequence of the *cheY-yrhK* operon (260 bp upstream of the *cheY-yrhK* translational start codon), *lamB-cheR* operon (239 bp), *yaaR-fliG2* operon (235 bp), *cheV-mogR* operon (203 bp), *hag1* gene (317 bp), *hag2* gene (610 bp), and *yjbJ-flgG* operon (316 bp) were amplified from HD73 genomic DNA with the primers P*cheY-yrhK*-F/P*cheY-yrhK*-R, P*lamB-cheR*-F/P*lamB-cheR*-R, P*yaaR-fliG2*-F/P*yaaR-fliG2*-R, P*cheV-mogR*-F/P*cheV-mogR*-R, P*hag1*-F/P*hag1*-R, P*hag2*-F/P*hag2*-R, P*yjbJ-flgG*-F/P*yjbJ-flgG*-R, respectively. These fragments of the promoter regions were digested with PstI and BamHI, followed by ligation into the linearized pHT304-18Z plasmid[35]. The recombinant 304P*cheY-yrhK*, 304P*lamB-cheR*, 304P*yaaR-fliG2*, 304P*cheV-mogR*, 304P*hag1*, 304P*hag2*, and 304P*yjbJ-flgG* plasmids were introduced into HD73 and Δ*cdsR*, resulting in HD(P*cheY-yrhK*), Δ*cdsR*(P*cheY-yrhK*), HD(P*lamB-cheR*), Δ*cdsR*(P*lamB-cheR*), HD(P*yaaR-fliG2*), Δ*cdsR*(P*yaaR-fliG2*), HD(P*cheV-mogR*), Δ*cdsR*(P*cheV-mogR*), HD(P*hag1*), Δ*cdsR*(P*hag1*), HD(P*hag2*), Δ*cdsR*(P*hag2*), HD(P*yjbJ-flgG*), and Δ*cdsR*(P*yjbJ-flgG*), respectively. These generated strains were validated by erythromycin resistance and PCR identification.

The β-galactosidase activities were measured as follows: the samples were collected from T_{-1} to T_6 (T_0 indicates the end of the exponential growth phase, and T_n indicates the number of hours before (-) or after T_0) at 1 h intervals. For each sample, 2 mL cultures from 100 mL were centrifuged (12,000×g, 1 min), and the pellets were stored at −40 °C before the test. For the determination of β-galactosidase activities, samples were resuspended in 0.5 mL of Z-buffer (0.06 M Na_2HPO_4, 0.04 M NaH_2PO_4, 0.01 M KCL, 1 mM $MgSO_4$, 1 mM dithiothreitol). The cells were disrupted with glass beads (0.1 mm; BioSpec; America) in a Mini Beadbeater-96 (BioSpec; America), and cell extract was obtained after centrifugation. Next, 0.7 mL of Z-buffer and 0.2 mL of 4 mg mL^{-1} 2-nitrophenyl β-D-galactopyranoside (sigma) for β-galactosidase assay were added to 100 μL of cell extract. The mixture was incubated at 37 °C, and the reaction was stopped by the addition of 0.5 mL of 1 M Na_2CO_3. Subsequently, the optical density of the reaction mixture was measured at 420 nm for the β-galactosidase assay. The protein content was determined using the Bio-Rad protein assay with bovine serum albumin as the standard. Specific activities are expressed in units of β-galactosidase per milligram of protein (Miller units). Miller units were calculated using the following formula: units = $(OD_{420}\times 1500)/T \times V \times$ protein

concentration. T is the reaction time (min) and V is the volume of sample added (μL). Values are reported as the mean and standard error of at least 3 independent assays.

Electrophoretic mobility shift assays

The expression and purification of CdsR-His protein were performed as previously described[19]. The promoter DNA fragment was PCR amplified from HD73 genomic DNA using specific primers labeled with a 5′-end FAM modification and confirmed by DNA sequencing. Electrophoresis mobility shift assays (EMSA) were performed to analyze the binding between CdsR protein and P*motAB1*, P*motAB2*, P*cheY-yrhK*, P*lamB-cheR*, P*yaaR-fliG2*, P*cheV-mogR*, P*hag1*, P*hag2*, P*yjbJ-flgG*, and P*lrgAB* promoter fragments. Briefly, the promoter DNA probe was incubated with different concentrations of purified CdsR at 25 °C for 30 min in binding buffer (10 mM Tris-HCl, 0.5 mM dithiothreitol, 50mM Nacl, 500 ng poly (dI: dC), pH 7.5 and 4% (v/v) glycerol) in a total volume of 20 μL. The DNA-protein mixtures were applied to non-denaturing 6.5% (w/v) polyacrylamide gels in TBE buffer (90 mM Tris-base, 90 mM boric acid, 2 mM EDTA, pH 8.0) for resolution of the complexes, using a Mini-PROTEAN system (Bio-Rad) at 170 V for 1 h. Signals were visualized directly from the gel with the FLA Imager FLA-5100 (Fujifilm).

Transcriptome sequencing analysis

The HD73 and Δ*cdsR* mutant strains were cultured in SSM at 30 °C and harvested at T0. Total RNA was extracted using the RNAprep Pure Bacteria Kit (aidlab, Beijing, China) to RNA sequencing (RNA-seq). The relative transcript abundance was calculated as fragments per kilobase of exon sequence per million mapped sequence reads (FPKM). Genes were considered differentially expressed only if they met the criteria of having an adjusted P-value ≤ 0.05 and absolute value of $\log_2$fold-change ≥ 1. The differentially expressed genes were subjected to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using an online gene function analysis tool. All RNA-Seq data were uploaded to the Gene Expression Omnibus database of the National Center for Biotechnology Information (accession no. GSE216307).

Data availability

Data are contained within the article. We have uploaded the RNA-seq data, which will be available in the NCBI Gene Expression Omnibus database with the accession no. GSE216307.

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References

1. Minamino, T., Kinoshita, M. & Structure Assembly, and function of Flagella responsible for bacterial locomotion. *EcoSal Plus* **11**, eesp00112023. https://doi.org/10.1128/ecosalplus.esp-0011-2023 (2023).
2. Tanimura, S. et al. ERK signaling promotes cell motility by inducing the localization of myosin 1E to lamellipodial tips. *J. Cell. Biol.* **214**, 475–489. https://doi.org/10.1083/jcb.201503123 (2016).
3. Huang, Z., Pan, X., Xu, N. & Guo, M. Bacterial chemotaxis coupling protein: structure, function and diversity. *Microbiol. Res.* **219**, 40–48. https://doi.org/10.1016/j.micres.2018.11.001 (2019).
4. Chaban, B., Hughes, H. V. & Beeby, M. The flagellum in bacterial pathogens: for motility and a whole lot more. *Semin. Cell Dev. Biol.* **46**, 91–103. https://doi.org/10.1016/j.semcdb.2015.10.032 (2015).
5. Mukherjee, S. & Kearns, D. B. The structure and regulation of flagella in *Bacillus subtilis*. *Annu. Rev. Genet.* **48**, 319–340. https://doi.org/10.1146/annurev-genet-120213-092406 (2014).
6. Davis, M. C., Kesthely, C. A., Franklin, E. A. & MacLellan, S. R. The essential activities of the bacterial sigma factor. *Can. J. Microbiol.* **63**, 89–99. https://doi.org/10.1139/cjm-2016-0576 (2017).
7. Chilcott, G. S. & Hughes, K. T. Coupling of flagellar gene expression to flagellar assembly in *Salmonella enterica* serovar typhimurium and *Escherichia coli*. *Microbiol. Mol. Biology Reviews: MMBR.* **64**, 694–708. https://doi.org/10.1128/mmbr.64.4.694-708.2000 (2000).
8. Cozy, L. M. et al. SlrA/SinR/SlrR inhibits motility gene expression upstream of a hypersensitive and hysteretic switch at the level of σ(D) in *Bacillus subtilis*. *Mol. Microbiol.* **83**, 1210–1228. https://doi.org/10.1111/j.1365-2958.2012.08003.x (2012).
9. Studholme, D. J. & Buck, M. The alternative sigma factor sigma(28) of the extreme thermophile *Aquifex aeolicus restores* motility to an *Escherichia coli fliA* mutant. *FEMS Microbiol. Lett.* **191**, 103–107. https://doi.org/10.1111/j.1574-6968.2000.tb09325.x (2000).
10. Cozy, L. M. & Kearns, D. B. Gene position in a long operon governs motility development in *Bacillus subtilis*. *Mol. Microbiol.* **76**, 273–285. https://doi.org/10.1111/j.1365-2958.2010.07112.x (2010).
11. Shin, S. & Park, C. Modulation of flagellar expression in *Escherichia coli* by acetyl phosphate and the osmoregulator OmpR. *J. Bacteriol.* **177**, 4696–4702. https://doi.org/10.1128/jb.177.16.4696-4702.1995 (1995).
12. Busenlehner, L. S., Pennella, M. A. & Giedroc, D. P. The SmtB/ArsR family of metalloregulatory transcriptional repressors: structural insights into prokaryotic metal resistance. *FEMS Microbiol. Rev.* **27**, 131–143. https://doi.org/10.1016/s0168-6445(03)00054-8 (2003).
13. Cavet, J. S., Graham, A. I., Meng, W. & Robinson, N. J. A cadmium-lead-sensing ArsR-SmtB repressor with novel sensory sites. Complementary metal discrimination by NmtR AND CmtR in a common cytosol. *J. Biol. Chem.* **278**, 44560–44566. https://doi.org/10.1074/jbc.M307877200 (2003).
14. Ren, S., Li, Q., Xie, L. & Xie, J. Molecular mechanisms underlying the function diversity of ArsR Family Metalloregulator. *Crit. Rev. Eukaryot. Gene Expr.* **27**, 19–35. https://doi.org/10.1615/CritRevEukaryotGeneExpr.2016018476 (2017).
15. Zhang, X. et al. A strong promoter of a non-cry gene directs expression of the cry1Ac gene in Bacillus thuringiensis. *Appl. Microbiol. Biotechnol.* **102**, 3687–3699. https://doi.org/10.1007/s00253-018-8836-5 (2018).
16. Gohar, M. et al. Two-dimensional electrophoresis analysis of the extracellular proteome of *Bacillus cereus* reveals the importance of the PlcR regulon. *Proteomics.* **2**, 784–791. https://doi.org/10.1002/1615-9861(200206)2:6<784::AID-PROT784>3.0.CO;2-R (2002).
17. Fagerlund, A. et al. SinR controls enterotoxin expression in *Bacillus thuringiensis* biofilms. *PloS One.* **9**, e87532. https://doi.org/10.1371/journal.pone.0087532 (2014).
18. Kearns, D. B., Chu, F., Branda, S. S., Kolter, R. & Losick, R. A master regulator for biofilm formation by *Bacillus subtilis*. *Mol. Microbiol.* **55**, 739–749. https://doi.org/10.1111/j.1365-2958.2004.04440.x (2005).
19. Zhang, X. et al. Cell death dependent on holins LrgAB repressed by a novel ArsR family regulator CdsR. *Cell. Death Discovery.* **10**, 173. https://doi.org/10.1038/s41420-024-01942-3 (2024).

20. Tjaden, B. De novo assembly of bacterial transcriptomes from RNA-seq data. *Genome Biol.* **16**, 1. https://doi.org/10.1186/s13059-014-0572-2 (2015).
21. Terahara, N., Namba, K. & Minamino, T. Dynamic exchange of two types of stator units in *Bacillus subtilis* flagellar motor in response to environmental changes. *Comput. Struct. Biotechnol. J.* **18**, 2897–2907. https://doi.org/10.1016/j.csbj.2020.10.009 (2020).
22. Bi, S. & Sourjik, V. Stimulus sensing and signal processing in bacterial chemotaxis. *Curr. Opin. Microbiol.* **45**, 22–29. https://doi.org/10.1016/j.mib.2018.02.002 (2018).
23. Smith, V. et al. MogR is a ubiquitous transcriptional repressor affecting motility, Biofilm formation and virulence in *Bacillus thuringiensis*. *Front. Microbiol.* **11**, 610650. https://doi.org/10.3389/fmicb.2020.610650 (2020).
24. Twine, S. M. et al. Motility and flagellar glycosylation in *Clostridium difficile*. *J. Bacteriol.* **191**, 7050–7062. https://doi.org/10.1128/jb.00861-09 (2009).
25. Guinebretière, M. H. et al. *Bacillus cytotoxicus* sp. nov. is a novel thermotolerant species of the *Bacillus cereus* Group occasionally associated with food poisoning. *Int. J. Syst. Evol. MicroBiol.* **63**, 31–40. https://doi.org/10.1099/ijs.0.030627-0 (2013).
26. Chauhan, S., Kumar, A., Singhal, A., Tyagi, J. S. & Krishna Prasad, H. CmtR, a cadmium-sensing ArsR-SmtB repressor, cooperatively interacts with multiple operator sites to autorepress its transcription in *Mycobacterium tuberculosis*. *FEBS J.* **276**, 3428–3439. https://doi.org/10.1111/j.1742-4658.2009.07066.x (2009).
27. Saha, R. P. et al. Metal homeostasis in bacteria: the role of ArsR-SmtB family of transcriptional repressors in combating varying metal concentrations in the environment. *Biometals: Int. J. role Metal ions Biology Biochem. Med.* **30**, 459–503. https://doi.org/10.1007/s10534-017-0020-3 (2017).
28. Xu, C. & Rosen, B. P. Dimerization is essential for DNA binding and repression by the ArsR metalloregulatory protein of *Escherichia coli*. *J. Biol. Chem.* **272**, 15734–15738. https://doi.org/10.1074/jbc.272.25.15734 (1997).
29. Zhi, F. et al. An ArsR transcriptional regulator *facilitates Brucella* sp. Survival via regulating self and outer membrane protein. *Int. J. Mol. Sci.* 22. https://doi.org/10.3390/ijms221910860 (2021).
30. Schaeffer, P., Millet, J. & Aubert, J. Catabolic repression of bacterial sporulation. *Proc. Natl. Acad. Sci. U.S.A.* **54**, 704–711. https://doi.org/10.1073/pnas.54.3.704 (1965).
31. Macaluso, A. & Mettus, A. M. Efficient transformation of *Bacillus thuringiensis* requires nonmethylated plasmid DNA. *J. Bacteriol.* **173**, 1353–1356. https://doi.org/10.1128/jb.173.3.1353-1356.1991 (1991).
32. Zhang, X. et al. A Novel Regulator PepR regulates the expression of dipeptidase gene pepV in Bacillus thuringiensis. *Microorganisms*. **12** https://doi.org/10.3390/microorganisms12030579 (2024).
33. Sambrook, B. J. & Russell, D. W. Molecular cloning. A laboratory manual. 3rd edn. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2015).
34. Lereclus, D., Arantes, O., Chaufaux, J. & Lecadet, M. Transformation and expression of a cloned delta-endotoxin gene in *Bacillus thuringiensis*. *FEMS Microbiol. Lett.* **51**, 211–217. https://doi.org/10.1016/0378-1097(89)90511-9 (1989).
35. Agaisse, H. & Lereclus, D. Structural and functional analysis of the promoter region involved in full expression of the *cryIIIA* toxin gene of *Bacillus thuringiensis*. *Mol. Microbiol.* **13**, 97–107. https://doi.org/10.1111/j.1365-2958.1994.tb00405.x (1994).

Author contributions

F.S. and X.Z. designed the experiments. X.Z., Y.C., T. Y., and H.W. performed the experiments. Y.L. and L.G. gave good advice on RNA extraction experiments. X.Z. and F.S. analyzed the results. X.Z. wrote the manuscript. F.S., Q.P., and J.L. gave good advice on the writing of the manuscript. F.S. revised the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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