Insights into sRNA Genes Regulated by Two-Component Systems in the Bacillus cereus Group

Article *in* Current Bioinformatics · September 2015

DOI: 10.2174/1574893610041509221510 28

CITATION
1

READS
30

6 authors, including:

Qing Tang
Huazhong Agricultural University
10 PUBLICATIONS **24** CITATIONS
SEE PROFILE

Yaxi Wang
Texas A&M University
2 PUBLICATIONS **5** CITATIONS
SEE PROFILE

Jieping Wang
Huazhong Agricultural University
27 PUBLICATIONS **155** CITATIONS
SEE PROFILE

Jin He
Huazhong Agricultural University
88 PUBLICATIONS **694** CITATIONS
SEE PROFILE

Insights into sRNA Genes Regulated by Two-Component Systems in the *Bacillus cereus* Group

Han Mei[1], Qing Tang[1], Xinfeng Li[1], Yaxi Wang[1], Jieping Wang[2] and Jin He[*,1]

[1]*State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, P.R. China*

[2]*Agricultural Bio-Resource Institute, Fujian Academy of Agricultural Sciences, Fuzhou, Fujian 350003, P.R. China*

Jin He

Abstract: Two-component systems (TCSs) and small regulatory RNAs (sRNAs) form dense regulatory networks in bacteria. To expand the known repertoire of TCS regulons in the *Bacillus cereus* group, we employed an *in silico* strategy to identify sRNA genes that might be regulated by response regulators (RRs) of TCS. Using the whole genomes of 21 fully sequenced strains of the *B. cereus* group, we identified 12 different types of novel sRNA genes. Using transcriptome data from *B. thuringiensis* CT-43, we confirmed the independent transcription of both the sRNA_bc4 gene and the sRNA_bc6 gene. Furthermore, the sRNA_bc6 and sRNA_bc12 genes were demonstrated to exist exclusively in the *B. cereus* group and thus have the potential to act as molecular markers. Finally, we modified the recognition motifs of PhoP and YclJ in the *B. cereus* group. These results significantly contribute to our understanding of TCS regulons in bacteria.

Keywords: *Bacillus cereus* group, bioinformatics, motif-based search, response regulator (RR), RNA-seq, small regulatory RNA (sRNA), transcriptome, two-component system (TCS).

1. INTRODUCTION

Species in the *Bacillus cereus* group, including *B. anthracis*, *B. cereus*, *B. cytotoxicus*, *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis* and *B. weihenstephanensis* [1], share many genotypic and phenotypic properties and are capable of forming highly resistant dormant endospores under unfavorable conditions [2]. Their life cycle can be divided into vegetative growth and sporulation, which exhibit complex and precise features in spatio-temporal regulation and differentiation processes. While living in fluctuating environments, these organisms employ sophisticated signal transduction systems and exhibit robust capabilities with regard to regulation of metabolic processes.

Signal transduction systems in bacteria are mainly comprised of second messenger-mediated signal pathways, quorum sensing systems, chemotaxis receptors, anti-σ:σ factor pairs, Ser/Thr protein kinases and phosphatases, and two-component signal transduction systems (TCSs) [3-5]. Among these signaling systems, TCSs are especially significant as they regulate the majority of physiological processes, including bacterial growth, protein synthesis, chemotaxis, osmoregulation, sporulation, nutrient (e.g. nitrogen and phosphorus) metabolism, biosynthesis of secondary metabolites, virulence of pathogens, biofilm formation, and quorum sensing [6]. A TCS typically consists of a histidine kinase (HK) and a response regulator (RR). Generally, a TCS senses environmental changes and then transmits signals using the following mechanism: i) the input domain located in the N-terminus of a HK senses stimuli; ii) signal perception results in autophosphorylation of the histidine site located in the C-terminal catalytic and ATP binding domain of the HK; iii) signal perception further activates one or more cognate RR(s), leading to phosphorylation of the aspartate site within the N-terminal receiver domain; and iv) finally, the activated RR acts as a transcription factor to regulate diverse genes [4, 7]. Different RRs can be classified into six categories according to their downstream targets and functions: DNA binding (63%), RNA binding (1%), enzymatic (13%), stand alone (17%), protein binding (3%), and others (3%) [8]. Thus, binding to DNA to either activate or suppress gene expression is the main function of RR-handling signal transduction processes. Recently, multiple studies have indicated that besides activating protein-encoding genes, RRs can also activate the transcription of genes encoding small regulatory RNAs (sRNAs) [9-11].

Bacterial sRNAs are small (50-600 nucleotides) non-coding RNA molecules that are highly structured and contain several stem loops [12]. sRNAs have been shown to participate in a number of physiological processes in bacterial cells using a variety of mechanisms [13, 14]. Although they have been shown to play a role in the transcriptional initiation and elongation of mRNAs, sRNAs function mainly at the post-transcriptional level, controlling target gene expression by regulating the stability and subsequent translation of target mRNA [13, 15]. Moreover, they can influence the physiological function of proteins at the post-translational level [16, 17]. In response to stimuli, sRNAs have been shown to regulate various cellular processes, including the response to nutritional starvation,

*Address correspondence to this author at the College of Life Science and Technology, Huazhong Agricultural University, No. 1 Shizishan Street, Wuhan, Hubei 430070, P.R. China; Tel: +86 27 87280670; Fax: +86 27 87280670; E-mail: hejin@mail.hzau.edu.cn

2212-392X/15 $58.00+.00

quorum sensing, membrane pressure, and oxidative stress [15].

sRNAs and TCSs are known to form dense and complex regulatory networks [11]. Through RRs, many TCSs regulate the synthesis of sRNA genes, which, in turn, controls expression of target genes. Initially, TCS-mediated trans-encoded sRNAs were found to pair with mRNAs or directly bind to target proteins. Later on, cis-encoded antisense RNAs were reported as a new class of sRNAs regulated by TCSs [11]. These interconnected networks are typically involved in the regulation of complex physiological processes, such as quorum sensing, biofilm formation, and virulence [16]. In addition, sRNAs are able to regulate expression of TCS genes, additionally elevating the complexity of whole regulatory networks [11].

Uncovering the link between TCSs and sRNAs will help us to better understand regulation of gene expression. Thus, we sought to identify sRNA genes regulated by RRs in the *B. cereus* group. We employed an *in silico* strategy to predict sRNA genes regulated by RRs in whole genomes of 21 fully sequenced strains from the *B. cereus* group (Supplementary Table **S1**). By searching for RRs specifically recognized motifs followed by Rho-independent transcription terminators in the intergenic regions (IGRs), we identified 12 different types of novel sRNA genes. Finally, we sequenced *B. thuringiensis* of CT-43 and used transcriptome data to independently confirm transcription of some of these sRNA genes.

2. MATERIALS AND METHODS

2.1. Bacterial Strains and Culture Conditions

The *B. thuringiensis* subsp. *chinensis* strain CT-43 [18] was grown at 28°C, with shaking at 200 rpm in liquid GYS medium [19], comprised of $(NH_4)_2SO_4$, 2 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; $ZnSO_4 \cdot 7H_2O$, 0.005 g; $MnSO_4 \cdot 4H_2O$, 0.05 g; $CaCl_2$, 0.08 g; $CuSO_4 \cdot 5H_2O$, 0.005g; $FeSO_4 \cdot 7H_2O$, 0.0005g; K_2HPO_4, 0.5g; glucose, 1.0 g; and yeast extract 2.0 g/L (pH 7.4).

2.2. Quantitative Transcriptomics (RNA-seq)

2.2.1. RNA Isolation and mRNA Purification

Two biological replicate cell samples were collected at 7 h (mid-exponential growth phase), 9 h (early-stationary growth phase), 13 h (mid-stationary growth phase (sporulation)) and 22 h (attenuative phase (spore maturation and mother cell lysis phase)). Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Total RNA was dissolved in 200 μL of RNase-free water and the final concentration was determined using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Total RNA was incubated with 10 U of DNase I (Ambion, Austin, TX, USA) at 37ºC for 1 h and the volume was elevated to 250 μL by adding nuclease-free water. Messenger RNA underwent further purification by depleting ribosomal RNA and tRNA using the Terminator™ 5'-phosphate-dependent exonuclease (Epicenter, Madison, WI, USA). The final RNA samples were quantitated using a DU800 spectrophotometer (Beckman Coulter, Fullerton, CA, USA).

2.2.2. cDNA Synthesis and Illumina Sequencing

Double-stranded cDNA was generated using the RNA-Seq Library Preparation Kit (Gnomegen, San Diego, CA, USA) and sequenced on an Illumina Genome Analyzer IIx (San Diego, CA, USA).

2.3. Summary of RNA-Seq Data

The transcriptome data was obtained by RNA-seq using the Illumina Genome Analyzer IIx sequencing platform. After removing low-value sequence reads, the average length of the remaining reads was 110 nucleotides, and the total numbers of the remaining reads were 926,755, 1,096,665, 577,810 and 1,493,721 in average in the libraries of 7 h, 9 h, 13 h and 22 h, respectively. Thus, the sequencing coverage of the four growth phases was 10-fold to 27-fold (compared to the genome length), reflecting a satisfactory sequencing depth of each sample. Detailed statistical analyses have been described previously [20].

2.4. Identification of RR Specifically Recognizing sRNA Genes

The sRNA gene identification workflow was shown as Fig. (**1**). 21 strains with of *B. cereus* group whose whole genome sequences are available in the NCBI data were selected (Supplementary Table **S1**). In addition, to determine whether some genes are widespread in bacilli other than the *B. cereus* group, we selected the *Bacillus* species with whole-genome sequences available at NCBI as follows: *B. amyloliquefaciens*, *B. atrophaeus*, *B. cellulosilyticus*, *B. clausii*, *B. coagulans*, *B. halodurans*, *B. licheniformis*, *B. megaterium*, *B. pseudofirmus*, *B. pumilus*, *B. selenitireducens*, *Bacillus.* sp. *JS and B. subtilis*.

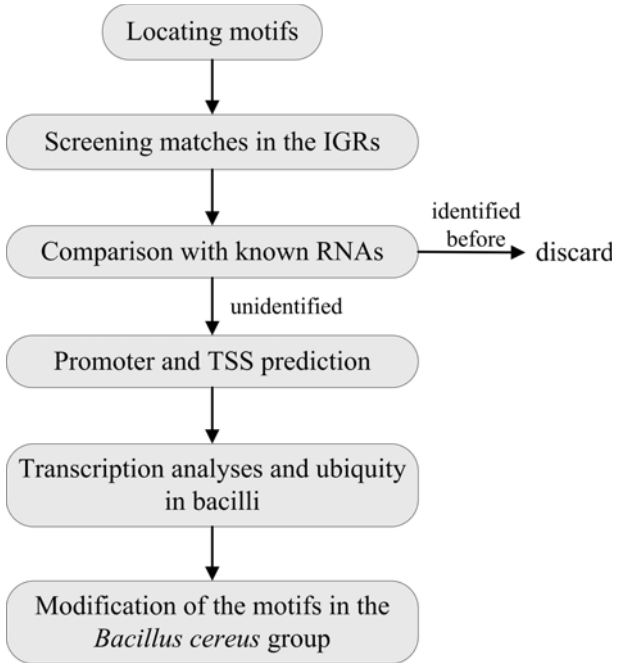

Fig. (1). Graphical representation of sRNA identification workflow.

2.4.1. Location of RR Recognition Motifs in Whole Genomes

Custom scripts programmed using Perl language were used to locate the coordinates of motifs in whole genomes and are attached as supplementary data (Supplementary perl scripts).

2.4.2. Screening Matches Situated in the IGRs

Rho-independent terminators were predicted in whole genomes using TransTermHP (http://transterm.cbcb.umd.edu/). Perl scripts were employed to ensure the motifs and the following independent terminators were both located in IGR. We defined:

(a) The start and end coordinates of a matched motif as motif_start and motif_end, respectively;

(b) Relative to a motif_end, the start coordinate of the nearest open reading frame (ORF) was defined as orf_start, with the distance defined as orf_distance;

(c) Relative to a motif_start, the end coordinate of the nearest ORF was defined as orf_end;

(d) Relative to a motif_end, the distance to nearest terminator was defined as term_distance.

In the positive strand, restrictions that orf_start must be less than orf_end and term_distance must be less than orf_distance were used to sift sRNA genes located in IGRs, and vice versa in the negative strand.

2.5. Comparison with Known RNAs

The sequences of all 12 types of sRNA genes were searched against the Rfam database (http://rfam.sanger.ac.uk/) to determine whether these sRNA were novel.

2.6. Promoter and Transcription Start Site (TSS) Prediction

We submitted the sequence around the motif to DBTBS (http://dbtbs.hgc.jp/), and visually inspected whether the sigma factor recognition site appeared around the motif.

2.7. Sequence Characteristics, Transcription Analyses, and Ubiquity in Bacilli

The length of a sRNA gene was calculated from the predicted TSS to the terminator. sRNAs were considered the same if at least 98% of the nucleotides were identical. The sequenced reads are non-directional. To more clearly distinguish a sRNA gene from the untranslated regions of its adjacent genes, we regarded the sequence from its upstream gene to downstream gene as a whole. Transcription of this whole was first evaluated by the number of mapped reads using Blastn (E=0.00001). Because the length of a sRNA gene was estimated, no RPKM value was calculated. We developed another Perl script to count the mapped frequency of each base. Origin 8.0 was used to draw the mapped frequencies of all bases. In cases where a few reads were mapped to a sRNA gene, we manually annotated the TSS in a progressive (successive approximation) manner [21]. In addition, we searched certain sRNA genes in the *Bacillus* species annotated by NCBI as described above.

2.8. Modification of RR Recognition Motifs

In cases when a sRNA gene was scattered in a few strains of the *B. cereus* group, we used Blastn to determine if the sRNA gene was present in the remaining strains of the *B. cereus* group members. In general, we found that sRNA genes that could not be detected in the first round were due to either motif differences between strains or due to the difficulty in predicting terminators in the remaining strains. Thus, we compared the motifs from available strains and used these to derive a new sequence motif.

3. RESULTS

3.1. sRNA Genes Regulated by the CiaH-CiaR (HK-RR) TCSs

CiaH-CiaR is a TCS that pleiotropically affects virulence, β-lactam resistance, autolysis, and competence regulation [4, 22, 23]. In *Streptococcus*, Marx *et al*. have found that amino acid sequences in the CiaR recognition helix are well conserved [24]. The authors provided experimental evidence that all streptococcal CiaR regulators can bind to the recognition motif (NTTAAG-n5-(A/T)TTAAG). Based on this, they found 40 different types of sRNA genes in *Streptococcus* that regulated by the CiaR. Until now, CiaR was only annotated in *B. cereus* G9241 (http://www.ncbi.nlm.nih.gov/protein/ZP_00237866.1) among the *B. cereus* group. We demonstrate its existence in all of the 21 selected strains of the *B. cereus* group using Blastp. We find that the recognition helix of CiaRs in the *B. cereus* group is highly similar to that in *Streptococcus pneumoniae* R6 (Supplementary Tables **S2** and **S3**). Therefore, we searched the conserved CiaR recognition motif in the genomes of the *B. cereus* group. Three types of sRNA genes were identified: the sRNA_bc1 gene was found in five strains; the sRNA_bc2 gene was detected only in *B. anthracis* G9842; and the sRNA_bc3 gene was found in 10 strains (Table **1**). Take the sRNA_bc3 gene in *B. anthracis* A0248 for instance, it was located within the encoding region of the *BAA_5044* gene and it occupied an independent promoter and a weak terminator (Fig. **2**). The results indicate that the promoter region of the sRNA_bc3 gene possesses the conserved -10 region (GGGTAA) and -35 region (GTTTGTT) recognized by SigB (in response to general stress). In *Streptococcus*, the SigA recognition region TATAAT was adjacent to the CiaR recognition motif [24]. Here, in the promoter region of *BAA_5044* gene, we also found the TATAAT sequence was next to the CiaR recognition motif. Thus, we speculate that both the *BAA_5044* gene and the sRNA_bc3 gene are regulated by the CiaH-CiaR TCS. In addition, the *BAA_5044* gene could also be controlled by SigA and the sRNA_bc3 gene might be otherwise regulated by SigB in response to certain stimuli.

3.2. sRNA Genes Regulated by the GlnK-GlnL (HK-RR) TCS

In *B. subtilis*, *glsA* and *glnT* encode glutaminase and glutamine transporter, respectively. GlnK-GlnL positively

Table 1. List of sRNA genes regulated by CiaR.

Strains	csRNA	Start[a]	End[b]	Terminator[c]	Sigma Factor
B. cereus biovar *anthracis* str. CI	sRNA_bc3	4502605	4502589	-[d]	SigB
B. thuringiensis BMB171	N/A				
B. anthracis str. 'Ames Ancestor'	sRNA_bc1	942464	942480	109	
	sRNA_bc3	4566236	4566220	270	SigB
B. anthracis str. Ames	sRNA_bc1	942463	942479	109	
	sRNA_bc3	4566109	4566093	-	SigB
B. cereus ATCC 14579	N/A				
B. cereus AH820	sRNA_bc3	4630801	4630785	-	SigB
B. cereus AH187	N/A				
B. thuringiensis str. Al Hakam	sRNA_bc3	4572837	4572821	-	SigB
B. thuringiensis serovar *konkukian* str. 97-27	N/A				
B. anthracis str. Sterne	sRNA_bc1	942358	942374	109	
	sRNA_bc3	4567329	4567313	-	SigB
B. cereus ATCC 10987	N/A				
B. anthracis str. A0248	sRNA_bc1	942364	942380	109	
	sRNA_bc3	4566136	4566120	270	SigB
B. anthracis str. CDC 684	sRNA_bc1	3296482	3296466	109	
	sRNA_bc3	4568467	4568451	-	SigB
B. cereus 03BB102	sRNA_bc3	4576902	4576886	-	SigB
B. cereus Q1	N/A				
B. cereus G9842	sRNA_bc2	2691519	2691503	91	SigG
B. cereus B4264	N/A				
B. weihenstephanensis KBAB4	N/A				
B. cereus NVH 391-98	N/A				
B. cereus E33L	sRNA_bc3	4627657	4627641	-	SigB
B. thuringiensis CT-43	N/A				

[a]The start coordinate of the motif.
[b]The end coordinate of the motif.
[c]The distance between the end coordinate of the motif and the start coordinate of the terminator.
[d]Terminator could not be predicted by TranstermHP.

controls the expression of the *glsA-glnT* operon, thereby participating in the regulation of glutamine utilization [25]. It has been reported that GlnL specifically recognizes a conserved motif (TTTTGT-n4-TTTTGT) in the promoter region of *glsA-glnT* [25]. We first searched in the *B. cereus* group genomes without mismatch permitted, but no sRNA gene was found. When one mismatch was enabled in each repeat of the sequence motif, a considerable number of results were obtained. Most of these were located in the untranslated regions of adjacent genes. To remove the false positives, we specifically used transcriptome data from *B. thuringiensis* CT-43 and discovered the sRNA_bc4 gene (Fig. **3A**, **B**). Furthermore, in *B. thuringiensis* CT-43, we found the sRNA_bc5 gene by observing the existence of a two-tandem motif in its promoter region. Thus, only two sRNA genes were identified in *B. thuringiensis* CT-43 (Table **2**, Fig. **3C**).

Independent transcription was detected in the sRNA_bc4 gene (Supplementary Table **S4**). For the sRNA_bc5 gene, no transcript was detected, implying that it might require a specific stimulus. We observed the existence of two GlnL recognition motifs (four repeats) in tandem and a credible SigB recognition site in the promoter region of the sRNA_bc5 gene, implying that there is likely a stringent regulation of the sRNA_bc5 gene in response to a specific stimulus.

3.3. sRNA Gene Regulated by the PhoR-PhoP (HK-RR) TCS

PhoR-PhoP typically governs the adaption to phosphate limitation, and it also regulates genetic competence [26], virulence [27], and sensing/resistance to oxidative stress [28, 29]. The consensus sequence motif (TT(A/T/C)ACA-n3~7-

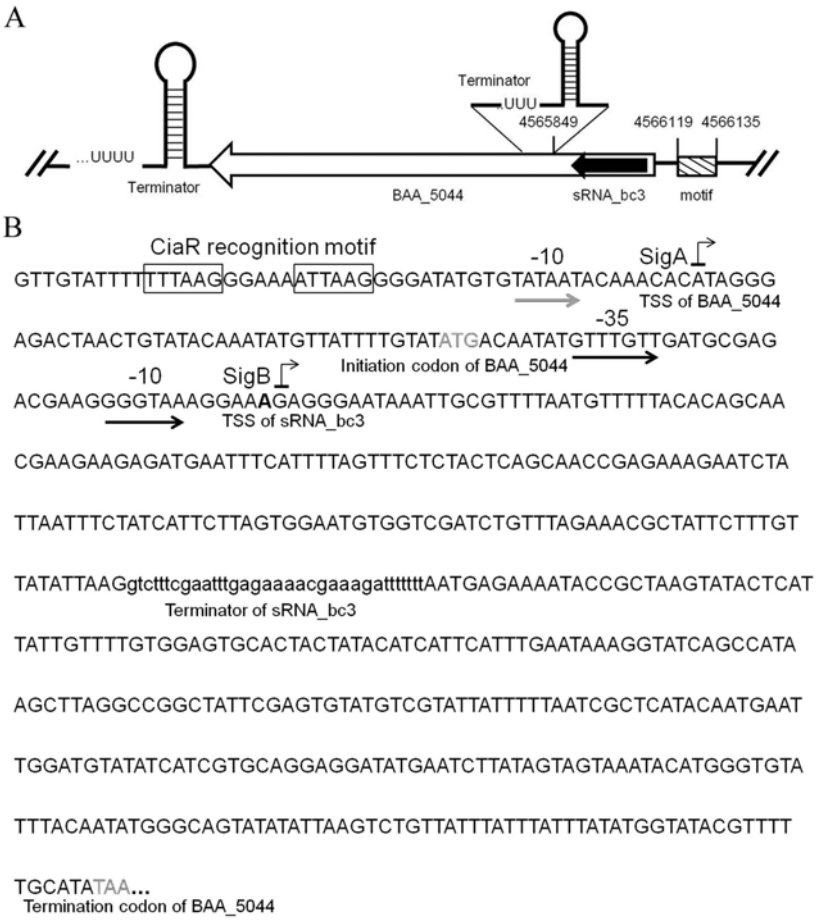

Fig. (2). Features of the sRNA_bc3 gene and *BAA_5044* gene. (**A**) Schematic structure of the sRNA_bc3 and BAA_5044 mRNA. (**B**) Nucleotide sequences of the sRNA_bc3 gene and *BAA_5044* gene. The nucleotide sequences in the box indicate the CiaR recognition motif. The gray arrows and black arrows indicate the SigA and SigB recognition sites, respectively. The bent arrows indicate the corresponding transcription start sites. The lowercase letters indicate sRNA_bc3 transcription terminator. The grey ATG and TAA indicate the initiation and termination codons of *BAA_5044* gene, respectively.

Table 2. List of sRNA genes regulated by GlnL in *B. thuringiensis* CT-43.

gsRNA	Start[a]	End[b]	Terminator[c]	Annotation	Sigma Factor
sRNA_bc4	3150208	3150193	432	Novel sRNA	SigA
sRNA_bc5	4737615	4737600	109	Novel sRNA	SigB
	4737584	4737569	78		

[a]The start coordinate of the motif.
[b]The end coordinate of the motif.
[c]The distance between the end coordinate of the motif and the start coordinate of the terminator.

TT(A/T/C)ACA)) recognized by PhoP has been reported in several species including *B. subtilis* and *Mycobacterium tuberculosis* [30]. We scanned the 21 selected genomes with this sequence motif and obtained a number of matches. Extraordinarily, PhoP recognized two tandem motifs to operate gene regulation (four repeats): one motif (two repeats) was for sequence recognition but was insufficient for expression of downstream genes [30]. We re-scanned the genomes with two tandem motifs (four repeats) using a distance of 3~7 nucleotides without mismatch and obtained a result showing great uniformity. Only one gene for the sRNA_bc6 was found in 12 strains (Table **3**). After further search by Blastn using the sequence of sRNA_bc6 gene, we found a nearly identical sequence in the genomes of eight other strains (see Materials and Methods, Modification of RR recognition motifs); the only difference lies in the first base of the fourth repeat of the motif (Table **4**). As an example, the sequence characteristics of the sRNA_bc6 gene in *B. thuringiensis* CT-43 are displayed in Fig. (**4A**). Moreover, in *B. thuringiensis* CT-43, transcription of the sRNA_bc6 gene was detected at 13 h (Fig. **4B**, Supplementary Table **S5**) and the putative TSS was approached according to the transcription tendency (Fig. **4A**). Comparing the motif in the 20 strains, it was suggested that in the *B. cereus* group, the PhoP recognition motif could be revised as (TT(A/T/C)ACA-n3~7-(T/C)T(A/T/C)ACA) (Table **4**).

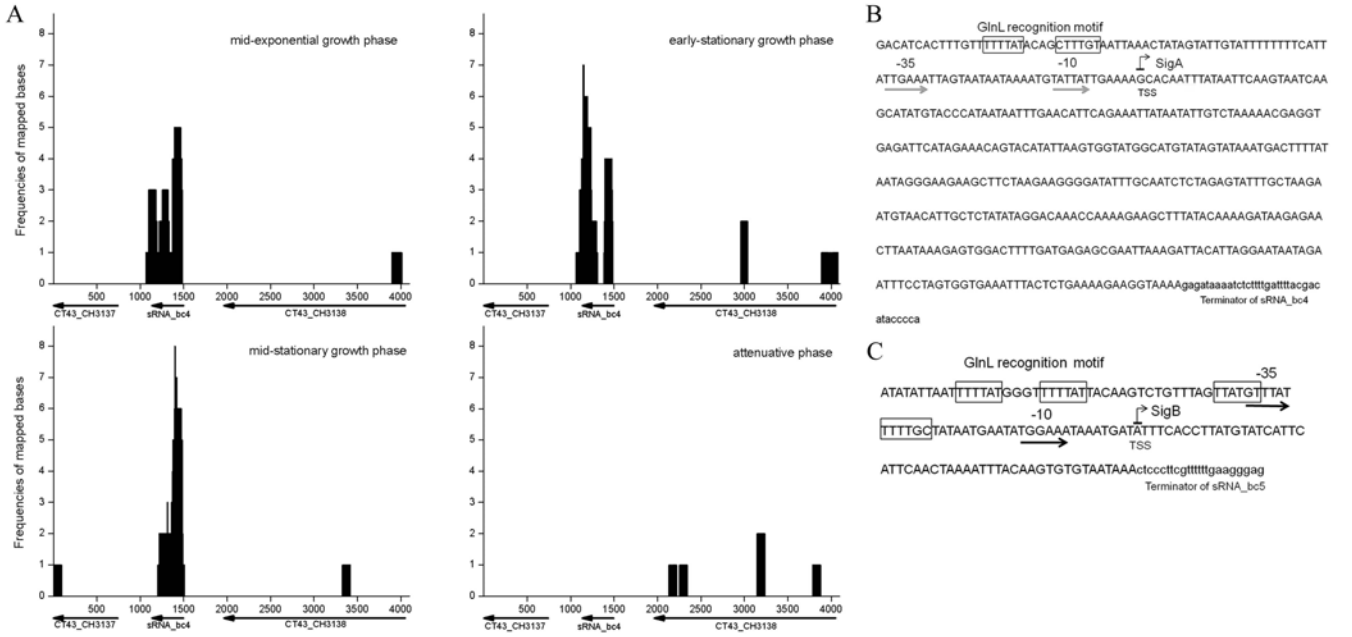

Fig. (3). Transcription and sequence characteristics of the sRNA_bc4 gene. (**A**) Transcription of the sRNA_bc4 gene and adjacent genes at the four phases. (**B**) Nucleotide sequences of the sRNA_bc4 gene. The nucleotide sequences in the box indicate the GlnL recognition motif. The gray arrows indicate the SigA recognition sites. The bent arrows indicate the corresponding transcription start sites. The lowercase letters indicate the sRNA_bc4 transcription terminator. (**C**) Nucleotide sequences of the sRNA_bc5 gene. The nucleotide sequences in the box indicate the GlnL recognition motif. The black arrows indicate the SigB recognition sites. The bent arrows indicate the corresponding transcription start sites. The lowercase letters indicate the sRNA_bc5 transcription terminator.

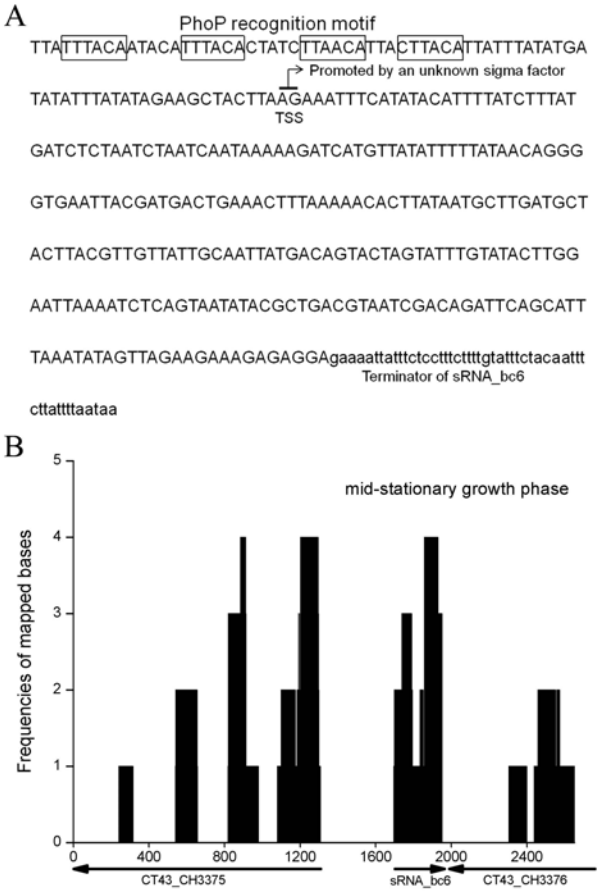

Fig. (4). Transcription and sequence characteristics of the sRNA_bc6 gene. (**A**) Nucleotide sequences of the sRNA_bc6 gene. The nucleotide sequences in the box indicate the PhoP recognition sequence motif. The bent arrows indicate the putative TSS approached by the transcriptome data. The lowercase letters indicate sRNA_bc6 transcription terminator. (**B**) Transcription of the sRNA_bc6 gene and adjacent genes at 13 h.

Table 3. List of sRNA genes regulated by PhoP.

Strains	psRNA	Start[a]	End[b]	Terminator[c]
B. cereus biovar *anthracis* str. CI	sRNA_bc6	3206219	3206255	267
B. thuringiensis BMB171	sRNA_bc6	3326038	3326074	-[d]
B. anthracis str. 'Ames Ancestor'	sRNA_bc6	3216626	3216662	267
B. anthracis str. Ames	sRNA_bc6	3216499	3216535	267
B. cereus ATCC 14579	sRNA_bc6	3401576	3401612	-
B. cereus AH820	sRNA_bc6	3296417	3296453	267
B. cereus AH187	sRNA_bc6	3230389	3230425	267
B. thuringiensis str. Al Hakam	sRNA_bc6	3282900	3282936	266
B. thuringiensis serovar *konkukian* str. 97-27	sRNA_bc6	3289019	3289055	267
B. anthracis str. Sterne	sRNA_bc6	3217192	3217228	267
B. cereus ATCC 10987	sRNA_bc6	3216380	3216416	266
B. anthracis str. A0248	sRNA_bc6	3216526	3216562	267
B. anthracis str. CDC 684	sRNA_bc6	1022130	1022094	267
B. cereus 03BB102	sRNA_bc6	3286041	3286077	266
B. cereus Q1	sRNA_bc6	3195019	3195055	267
B. cereus G9842	sRNA_bc6	3328190	3328226	-
B. cereus B4264	sRNA_bc6	3332241	3332277	-
B. weihenstephanensis KBAB4	sRNA_bc6	3200575	3200611	-
B. cereus NVH 391-98	N/A			
B. cereus E33L	sRNA_bc6	3285186	3285222	267
B. thuringiensis CT-43	sRNA_bc6	3386964	3387000	-

[a]The start coordinate of the motif.
[b]The end coordinate of the motif.
[c]The distance between the end coordinate of the motif and the start coordinate of the terminator.
[d]Terminator could not be predicted by TranstermHP.

Table 4. Sequence similarity of the PhoP recognition sequence motif in different strains.

Strains	PhoP Recognition Sequence Motif[a]
B. cereus biovar *anthracis* str. CI	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. thuringiensis BMB171	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-CTTACA....**
B. anthracis str. 'Ames Ancestor'	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. anthracis str. Ames	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. cereus ATCC 14579	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-CTTACA....**
B. cereus AH820	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. cereus AH187	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. thuringiensis str. Al Hakam	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. thuringiensis serovar *konkukian* str. 97-27	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. anthracis str. Sterne	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. cereus ATCC 10987	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. anthracis str. A0248	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. anthracis str. CDC 684	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. cereus 03BB102	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. cereus Q1	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. cereus G9842	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-CTTACA....**
B. cereus B4264	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-CTTACA....**
B. weihenstephanensis KBAB4	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. cereus E33L	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-TTTACA....**
B. thuringiensis CT-43	**...TTTACA-n5-TTTACA-n5-TTAACA-n3-CTTACA....**

Using Blastp, we found that PhoR-PhoP is widespread among genomes of the *Bacillus* species available from NCBI, but the sRNA_bc6 gene was only identified in the *B. cereus* group. Even though the function of sRNA_bc6 is unknown, sRNA_bc6 gene might be regulated by PhoP specifically in the *B. cereus* group.

3.4. sRNA Genes Regulated by the WalK-WalR (HK-RR) TCS

In bacilli, cross-talk between the PhoP-PhoR TCS and the essential WalK-WalR (also named as YycF-YycG) (HK-RR) TCS has been reported [31, 32]. To date, a number of genes regulated by WalK-WalR have been identified, including cell wall metabolism, oxidative stress and anaerobiosis, antibiotic resistance, and virulence [33, 34].

WalR has been reported to recognize the sequence motif (TGT(A/T)A(A/T/C)-n5-TGT(A/T)A(A/T/C)) [35]. A search of the 21 genomes with this sequence identified five sRNA genes (Table **5**). The sRNA_bc7 gene appeared in 10 strains. Sequence analysis found that the sRNA_bc7 gene was transcribed through a SigA-dependent promoter (Fig. **5A**), which indicated that it might take part in metabolic pathways essential to life. Another prominent sRNA was sRNA_bc10. The gene for sRNA_bc10 appeared in six strains. The sRNA_bc10 gene was transcribed through a SigB-dependent promoter (Fig. **5B**). We thus speculated that sRNA_bc10 could be associated with adaption to certain stress stimuli, consistent with a requirement for the WalK-WalR TCS. We think that the ubiquity of certain sRNA genes can be explained by being required for specific metabolic processes. Further experimentation must be done to determine whether the remaining genes (sRNA_bc9 and sRNA_bc11) scattering in particular strains are functional.

3.5. sRNA Gene Regulated by the YclK-YclJ (HK-RR) TCS

In *B. subtilis*, YclJ modulates three operons (*yclHI*, *yngABC* and *ykcBC*) and two single genes (*yycA* and *yfjR*) [36]. Investigations based on sequence similarity have demonstrated that genes under control of YclJ are associated with following processes: (i) constitution of an ABC-type transporter with substrates unknown [37]; (ii) glycoprotein biosynthesis in eukaryotic cells; and (iii) N-linked oligosaccharide synthesis [38].

Ogura *et al*. reported that the sequence motif (TTCATANTTT-n-TTCATANTTT) is recognized by YclJ in *B. subtilis* [36]. When we scanned the 21 *B. cereus* genomes with this motif, no sRNA gene was found. On the other hand, both YrkP and YclJ belong to the OmpR family in which the recognition motifs are shared to some degree [34, 36]. YrkP has been shown to recognize a single binding sequence ((T/G)TCA(T/C)AAATT) [39]. Subsequently we re-searched with one mismatch allowed in each repeat of the motif. One gene for sRNA termed sRNA_bc12 appeared just once in *B. weihenstephanensis* KBAB4. We continued to search this sRNA gene using Blastn and found its existence in another 19 strains (Table **6**). However, in *B. thuringiensis* CT-43, the transcript of sRNA_bc12 gene could not be detected at any growth phases. Additionally, the sRNA_bc12 gene was only found in the *B. cereus* group among the *Bacillus* species annotated by NCBI.

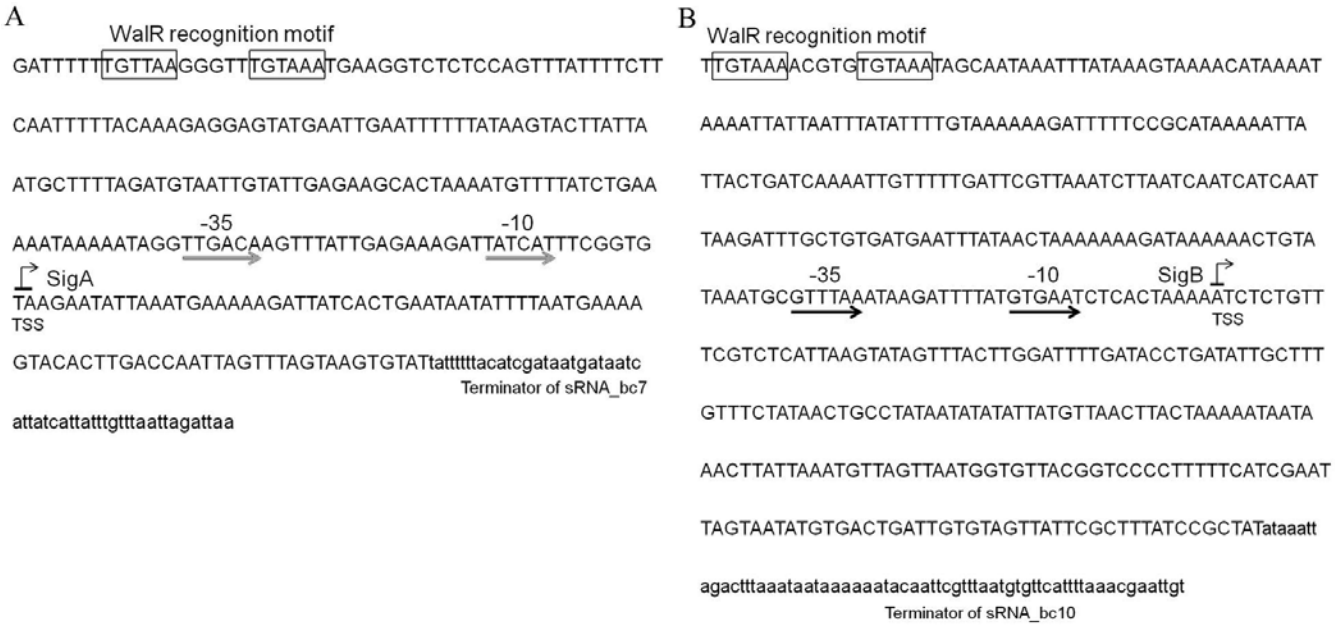

Fig. (5). Sequences of the sRNA_bc7 gene and the sRNA_bc10 gene. (**A**) Nucleotide sequences of the sRNA_bc7 gene. The nucleotide sequences in the box indicate the WalR recognition motif. The gray arrows indicate the SigA recognition sites. The lowercase letters indicate the sRNA_bc7 transcription terminator. (**B**) Nucleotide sequences of the sRNA_bc10 gene. The bent arrows indicate the corresponding transcription start sites. The nucleotide sequences in the box indicate the WalR recognition motif. The black arrows indicate the SigB recognition sites. The bent arrows indicate the corresponding transcription start sites. The lowercase letters indicate the sRNA_bc10 transcription terminator.

Table 5. List of sRNA genes regulated by WalR.

Strains	wsRNA	Start[a]	End[b]	Terminator[c]	Sigma Factor
B. cereus biovar *anthracis* str. CI	N/A				
B. thuringiensis BMB171	N/A				
B. anthracis str. 'Ames Ancestor'	sRNA_bc7	2201700	2201716	270	SigA
	sRNA_bc10	2508187	2508171	454	SigB
B. anthracis str. Ames	sRNA_bc7	2201576	2201592	270	SigA
	sRNA_bc10	2508063	2508047	454	SigB
B. cereus ATCC 14579	N/A				
B. cereus AH820	sRNA_bc7	2265534	2265550	270	SigA
B. cereus AH187	sRNA_bc8	1619713	1619729	342	SigH
B. thuringiensis str. Al Hakam	sRNA_bc7	2257554	2257570	270	SigA
B. thuringiensis serovar *konkukian* str. 97-27	sRNA_bc7	2222829	2222845	270	SigA
B. anthracis str. Sterne	sRNA_bc7	2201682	2201698	270	SigA
	sRNA_bc10	2508116	2508100	454	SigB
B. cereus ATCC 10987	sRNA_bc9	1493613	1493629	243	SigA
B. anthracis str. A0248	sRNA_bc7	2201600	2201616	270	SigA
	sRNA_bc10	2508087	2508071	454	SigB
B. anthracis str. CDC 684	sRNA_bc7	2037510	2037494	272	SigA
	sRNA_bc10	1730908	1730924	454	SigB
B. cereus 03BB102	sRNA_bc7	2264483	2264499	270	SigA
	sRNA_bc10	2593079	2593063	454	SigB
B. cereus Q1	N/A				
B. cereus G9842	sRNA_bc11	960810	960826	171	SigB
B. cereus B4264	N/A				
B. weihenstephanensis KBAB4	N/A				
B. cereus NVH 391-98	N/A				
B. cereus E33L	sRNA_bc7	2249864	2249880	271	SigA
B. thuringiensis CT-43	N/A				

[a]The start coordinate of the motif.
[b]The end coordinate of the motif.
[c]The distance between the end coordinate of the motif and the start coordinate of the terminator.

The sRNA_bc12 gene was detected in only one strain during the first round because either the motif occupied more than one mismatch or no terminator was predicted by TranstermHP (in *B. thuringiensis* CT-43, the relevant terminator could not be predicted). We extracted all of the sequence motifs of sRNA_bc12 gene in the 20 genomes to derive a new consensus sequence (TTGATANT(T/C)(T/C)-n-TTGATANTT(T/C)) (Table **7**). It is obvious that great similarities are shared between the YclJ and YrkP recognition sequences. Therefore, further experiments are needed to identify the OmpR family member that controls the sRNA_bc12 gene.

4. DISCUSSION

In prokaryotes, the ability to respond quickly to environmental changes, including changes in osmotic pressure and ionic strength, pH, temperature and concentrations of nutrients and hazardous substances are a prerequisite to survival [4, 11]. Bacterial TCSs play an indispensable role in converting these stimuli into intracellular responses.

4.1. Advantages of sRNA-mediated Metabolic Processes

Recently, increasing evidence indicates that sRNAs act as regulators of TCS signal networks, and sometimes TCSs themselves are regulated by sRNAs [11, 40]. The role that sRNAs play in regulatory networks has attracted considerable attention [11, 15]. The ubiquity of sRNAs is probably due to the following properties: i) advantages in regulation speed: compared to protein-regulating processes, sRNAs interact with pre-mRNA and are able to respond more quickly to sudden environmental changes; ii) low metabolic cost: sRNAs are composed of fewer nucleotides;

Table 6. List of sRNA genes regulated by YclJ.

Strains	ysRNA	Start[a]	End[b]	Terminator[c]
B. cereus biovar *anthracis* str. CI	sRNA_bc12	2146412	2146392	-[d]
B. thuringiensis BMB171	sRNA_bc12	2170381	2170361	-
B. anthracis str. 'Ames Ancestor'	sRNA_bc12	2171680	2171660	70
B. anthracis str. Ames	sRNA_bc12	2171556	2171536	70
B. cereus ATCC 14579	sRNA_bc12	2212598	2212578	-
B. cereus AH820	sRNA_bc12	2235466	2235446	-
B. cereus AH187	sRNA_bc12	2269126	2269106	70
B. thuringiensis str. Al Hakam	sRNA_bc12	2226955	2226935	70
B. thuringiensis serovar *konkukian* str. 97-27	sRNA_bc12	2191167	2191147	70
B. anthracis str. Sterne	sRNA_bc12	2171584	2171564	70
B. cereus ATCC 10987	sRNA_bc12	2254011	2253991	-
B. anthracis str. A0248	sRNA_bc12	2171580	2171560	-
B. anthracis str. CDC 684	sRNA_bc12	2067569	2067589	-
B. cereus 03BB102	sRNA_bc12	2233940	2233920	-
B. cereus Q1	sRNA_bc12	2224518	2224498	-
B. cereus G9842	sRNA_bc12	2157448	2157428	-
B. cereus B4264	sRNA_bc12	2198199	2198179	-
B. weihenstephanensis KBAB4	sRNA_bc12	2198934	2198914	69
B. cereus NVH 391-98	N/A			
B. cereus E33L	sRNA_bc12	2219091	2219071	-
B. thuringiensis CT-43	sRNA_bc12	2248547	2248527	-

[a]The start coordinate of the motif.
[b]The end coordinate of the motif.
[c]The distance between the end coordinate of the motif and the start coordinate of the terminator.
[d]Terminator could not be predicted by TranstermHP.

iii) evolutionary advantages: sRNAs are older "molecular clocks" than proteins; and iv) participation of the RNA chaperone Hfq: Hfq influences synthesis and degradation of certain transcripts and directly gives rise to densely interconnected regulatory networks by mediating the base-pairing of sRNAs and mRNAs [11, 15, 41, 42]. Importantly, the chromosome of the *B. cereus* group members always harbors two copies of *hfq* genes; in *B. thuringiensis* CT-43 [18], each of the two plasmids pCT83 and pCT72 contain an additional copy of *hfq*. These multiple sources of Hfq might imply a greater significance of sRNAs to the *B. cereus* group.

4.2. Molecular Marker and Phylogeny Analysis

Other than CiaH-CiaR, the remaining four TCSs have not been reported to regulate sRNA genes. In addition to identifying the sRNA_bc6 gene, we not only pulled out the two sequence motifs in tandem and confirmed the independent transcription of the sRNA_bc6 gene, but also verified its transcriptional start site and modified the motif recognized by PhoP in the *B. cereus* group. Kolstø *et al*. launched a systematic analysis of classification and evolution in the *B. cereus* group and a major criterion was whether some specific genes were present in the strains or not [43]. Among the sRNA genes identified in this research, the sRNA_bc6 gene existed only in the *B. cereus* group. This could be strong evidence that the *B. cereus* group shares great synteny. Thus, sRNA genes might be used as molecular markers to identify whether a new strain belongs in a certain category. Moreover, while the mutation, insertion or deletion of nucleotides could easily silence a protein, sRNAs could likely tolerate these changes to a much greater degree. Therefore, it is likely that, compared to proteins, sRNA genes might be more useful as molecular markers.

4.3. Regulatory Information of Nucleotide Sequences

Our work is based on specific RR recognition motifs and filtered using transcriptome data from *B. thuringiensis* CT-43. We identified 12 novel sRNA genes. In *B. thuringiensis* CT-43, the independent transcription of sRNA_bc4 gene and sRNA_bc6 gene was confirmed by the RNA-seq, and their TSSs were approached according to the enrichment of the transcription tendency. Additionally, the PhoP and YclJ recognition motifs in the *B. cereus* group were carefully revised. We proposed that sequence motifs are not only strings of nucleotides, but may also be responsible for connecting upstream *trans*-elements to downstream targets.

Table 7. Sequence similarity of the YclJ recognition sequence motif in different strains.

Strains	YclJ Recognition Sequence Motif[a]		
B. cereus biovar *anthracis* str. CI	**TTGATAnTCT**	**-n-**	**TTGATTnTTT**
B. thuringiensis BMB171	**TTGATAnTTC**	**-n-**	**TTGATTnTTT**
B. anthracis str. 'Ames Ancestor'	**TTGATAnTCT**	**-n-**	**TTGATTnTTT**
B. anthracis str. Ames	**TTGATAnTCT**	**-n-**	**TTGATTnTTT**
B. cereus ATCC 14579	**TTGATAnTTC**	**-n-**	**TTGATTnTTT**
B. cereus AH820	**TTGATAnTCT**	**-n-**	**TTGATTnTTT**
B. cereus AH187	**TTGATAnTTT**	**-n-**	**TTGATTnTTC**
B. thuringiensis str. Al Hakam	**TTGATAnTCT**	**-n-**	**TTGATTnTTT**
B. thuringiensis serovar *konkukian* str. 97-27	**TTGATAnTCT**	**-n-**	**TTGATTnTTT**
B. anthracis str. Sterne	**TTGATAnTCT**	**-n-**	**TTGATTnTTT**
B. cereus ATCC 10987	**TTGATAnTTT**	**-n-**	**TTGATTnTTC**
B. anthracis str. A0248	**TTGATAnTCT**	**-n-**	**TTGATTnTTT**
B. anthracis str. CDC 684	**TTGATAnTCT**	**-n-**	**TTGATTnTTT**
B. cereus 03BB102	**TTGATAnTCT**	**-n-**	**TTGATTnTTT**
B. cereus Q1	**TTGATAnTTT**	**-n-**	**TTGATTnTTC**
B. cereus G9842	**TTGATAnTTC**	**-n-**	**TTGATTnTTT**
B. cereus B4264	**TTGATAnTTC**	**-n-**	**TTGATTnTTT**
B. weihenstephanensis KBAB4	**TTGATAnTTT**	**-n-**	**TTGATAnTTT**
B. cereus E33L	**TTGATAnTCT**	**-n-**	**TTGATTnTTT**
B. thuringiensis CT-43	**TTGATAnTTC**	**-n-**	**TTGATTnTTT**

Recently, a number of transcription factors have been reported to recognize specific sequence motifs, and searches based on these motifs could probably reveal new components of metabolic pathways. As for the predicted sRNA genes in our work, further experimental investigation need to be done to determine if they are regulated by corresponding TCSs in the *B. cereus* group.

Once a motif-based search is carried out, considerations that different sequence motifs may vary in conservation should be taken. This determines whether the mismatch in nucleotide(s) should be allowed; whether the mismatch is occupied is highly correlated with both the number and the accuracy of predicted sRNA genes. For the CiaR recognition motif (NTTAAG-n5-(A/T)TTAAG), the exact amino acid sequences of the recognition helix have been clearly determined, so we permitted no mismatch during the search. In the process of uncovering sRNA genes regulated by GlnL, we used transcriptome data to verify the sRNA_bc4 gene. As for the other three TCSs in our work, all of the sequence motifs have been shown to be functional in *B. subtilis* in various experiments. Therefore, the exploration for TCS-sRNAs in this research could be applicable to *B. subtilis*, a *Bacillus* member closely related to the *B. cereus* group [44, 45]. Moreover, it is likely that some sRNA genes could act as molecular markers in the genus *Bacillus*.

CONCLUSION

We identified 12 different types of novel sRNA genes from the whole genomes of 21 fully sequenced strains in the *B. cereus* group. We additionally confirmed the independent transcription of sRNA_bc4 gene and sRNA_bc6 gene in *B. thuringiensis* CT-43 by RNA-seq. Furthermore, sRNA_bc6 and sRNA_bc12 genes were demonstrated to exist exclusively in the *B. cereus* group and therefore could probably act as molecular markers. Finally, we modified the recognition motifs of PhoP and YclJ in the *B. cereus* group. Our results would contribute to further understanding into signal transduction and TCS regulons in bacteria.

DATA DEPOSITION

RNAseq data were deposited in the NCBI'S GEO database (www.ncbi.nlm.nih.gov/geo/) under accession no. GSE39479.

ABBREVIATIONS

HK = Histidine kinase

IGR = Intergenic regions

ORF = Open reading frame

RR	=	Response regulator
sRNA	=	Small regulatory RNA
TCS	=	Two-component system
TSS	=	Transcription start site

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (grant 31270105) and the National High-tech R&D Program of China (863 Program, grant 2011AA10A205).

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

REFERENCES

[1] Ibrahim MA, Griko N, Junker M, Bulla LA. *Bacillus thuringiensis*: a genomics and proteomics perspective. Bioeng Bugs 2010; 1: 31-50.

[2] From C, van der Voort M, Abee T, Granum PE. Characterization of a spore-specific protein of the *Bacillus cereus* group. FEMS Microbiol Lett 2012; 331: 152-9.

[3] Gomelsky M. cAMP, c-di-GMP, c-di-AMP and now cGMP: bacteria use them all! Mol Microbiol 2011; 79: 562-5.

[4] Mascher T, Heintz M, Zahner D, Merai M, Hakenbeck R. The CiaRH system of *Streptococcus pneumoniae* prevents lysis during stress induced by treatment with cell wall inhibitors and by mutations in *pbp2x* involved in beta-lactam resistance. J Bacteriol 2006; 188: 1959-68.

[5] Ng WL, Bassler BL. Bacterial quorum-sensing network architectures. Annu Rev Genet 2009; 43: 197-222.

[6] Bourret BR, Silversmith ER. Two-component signal transduction. Curr Opin Microbiol 2010; 13: 113-5.

[7] Stock AM, Robinson VL, Goudreau PN. Two-component signal transduction. Annu Rev Biochem 2000; 69: 183-215.

[8] Gao R, Stock AM. Biological insights from structures of two-component proteins. Annu Rev Microbiol 2009; 63: 133-54.

[9] Bordi C, Lamy MC, Ventre I, *et al*. Regulatory RNAs and the HptB/RetS signalling pathways fine-tune *Pseudomonas aeruginosa* pathogenesis. Mol Microbiol 2010; 76: 1427-43.

[10] Coornaert A, Lu A, Mandin P, Springer M, Gottesman S, Guillier M. MicA sRNA links the PhoP regulon to cell envelope stress. Mol Microbiol 2010; 76: 467-79.

[11] Gopel Y, Gorke B. Rewiring two-component signal transduction with small RNAs. Curr Opin Microbiol 2012; 15: 132-9.

[12] Viegas SC, Arraiano CM. Regulating the regulators: How ribonucleases dictate the rules in the control of small non-coding RNAs. RNA Biol 2008; 5: 230-43.

[13] Vogel J, Gottesman S, Belasco J, Narberhaus F. Meeting report: Regulating with RNA in bacteria 2013. RNA Biol 2014; 11: 403-12.

[14] Liu JM, Camilli A. A broadening world of bacterial small RNAs. Curr Opin Microbiol 2010; 13(1): 18-23.

[15] Beisel CL, Storz G. Base pairing small RNAs and their roles in global regulatory networks. FEMS Microbiol Rev 2010; 34: 866-82.

[16] Babitzke P, Romeo T. CsrB sRNA family: sequestration of RNA-binding regulatory proteins. Curr Opin Microbiol 2007; 10: 156-63.

[17] Kay E, Dubuis C, Haas D. Three small RNAs jointly ensure secondary metabolism and biocontrol in *Pseudomonas fluorescens* CHA0. Proc Natl Acad Sci U S A 2005; 102: 17136-41.

[18] He J, Wang J, Yin W, *et al*. Complete genome sequence of *Bacillus thuringiensis* subsp. *chinensis* strain CT-43. J Bacteriol 2011; 193: 3407-8.

[19] Nickerson KW, St Julian G, Bulla LA, Jr. Physiology of sporeforming bacteria associated with insects: radiorespirometric survey of carbohydrate metabolism in the 12 serotypes of *Bacillus thuringiensis*. Appl Microbiol 1974; 28: 129-32.

[20] Wang J, Mei H, Zheng C, *et al*. The metabolic regulation of sporulation and parasporal crystal formation in *Bacillus thuringiensis* revealed by transcriptomics and proteomics. Mol Cell Proteomics 2013; 12: 1363-76.

[21] Sharma CM, Hoffmann S, Darfeuille F, *et al*. The primary transcriptome of the major human pathogen *Helicobacter pylori*. Nature 2010; 464: 250-5.

[22] Ibrahim YM, Kerr AR, McCluskey J, Mitchell TJ. Control of virulence by the two-component system CiaR/H is mediated *via* HtrA, a major virulence factor of *Streptococcus pneumoniae*. J Bacteriol 2004; 186: 5258-66.

[23] Sebert ME, Patel KP, Plotnick M, Weiser JN. *Pneumococcal* HtrA protease mediates inhibition of competence by the CiaRH two-component signaling system. J Bacteriol 2005; 187: 3969-79.

[24] Marx P, Nuhn M, Kovacs M, Hakenbeck R, Bruckner R. Identification of genes for small non-coding RNAs that belong to the regulon of the two-component regulatory system CiaRH in *Streptococcus*. BMC Genomics 2010; 11.

[25] Satomura T, Shimura D, Asai K, Sadaie Y, Hirooka K, Fujita Y. Enhancement of glutamine utilization in *Bacillus subtilis* through the GlnK-GlnL two-component regulatory system. J Bacteriol 2005; 187: 4813-21.

[26] Guo QG, Li SZ, Lu XY, Li BQ, Ma P. *PhoR/PhoP* two component regulatory system affects biocontrol capability of *Bacillus subtilis* NCD-2. Genet Mol Biol 2010; 33: 333-40.

[27] Ryndak M, Wang SS, Smith I. PhoP, a key player in *Mycobacterium tuberculosis* virulence. Trends Microbiol 2008; 16: 528-34.

[28] Darbon E, Martel C, Nowacka A, Pegot S, Moreau PL, Virolle MJ. Transcriptional and preliminary functional analysis of the six genes located in divergence of *phoR/phoP* in *Streptomyces lividans*. Appl Microbiol Biot 2012; 95: 1553-66.

[29] Pragai Z, Allenby NE, O'Connor N, *et al*. Transcriptional regulation of the phoPR operon in *Bacillus subtilis*. J Bacteriol 2004; 186: 1182-90.

[30] Eder S, Liu W, Hulett FM. Mutational analysis of the *phoD* promoter in *Bacillus subtilis*: implications for PhoP binding and promoter activation of Pho regulon promoters. J Bacteriol 1999; 181: 2017-25.

[31] Dubrac S, Bisicchia P, Devine KM, Msadek T. A matter of life and death: cell wall homeostasis and the WalKR (YycGF) essential signal transduction pathway. Mol Microbiol 2008; 70: 1307-22.

[32] Howell A, Dubrac S, Noone D, Varughese KI, Devine K. Interactions between the YycFG and PhoPR two-component systems in *Bacillus subtilis*: the PhoR kinase phosphorylates the non-cognate YycF response regulator upon phosphate limitation. Mol Microbiol 2006; 59: 1199-215.

[33] Dubrac S, Msadek T. Identification of genes controlled by the essential YycG/YycF two-component system of *Staphylococcus aureus*. J Bacteriol 2004; 186: 1175-81.

[34] Fabret C, Feher VA, Hoch JA. Two-component signal transduction in *Bacillus subtilis*: how one organism sees its world. J Bacteriol 1999; 181: 1975-83.

[35] Okajima T, Doi A, Okada A, Gotoh Y, Tanizawa K, Utsumi R. Response regulator YycF essential for bacterial growth. X-ray crystal structure of the DNA-binding domain and its PhoB-like DNA recognition motif. FEBS Letters 2008; 582: 3434-8.

[36] Ogura M, Tsukahara K, Tanaka T. Identification of the sequences recognized by the *Bacillus subtilis* response regulator YclJ. Arch Microbiol 2010; 192: 569-80.

[37] Quentin Y, Fichant G, Denizot F. Inventory, assembly and analysis of *Bacillus subtilis* ABC transport systems. J Mol Biol 1999; 287: 467-84.

[38] Langdon RH, Cuccui J, Wren BW. N-linked glycosylation in bacteria: an unexpected application. Future Microbiol 2009; 4: 401-12.

[39] Ogura M, Ohsawa T, Tanaka T. Identification of the sequences recognized by the *Bacillus subtilis* response regulator YrkP. Biosci Biotech Bioch 2008; 72: 186-96.

[40] Sonnleitner E, Abdou L, Haas D. Small RNA as global regulator of carbon catabolite repression in *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 2009; 106: 21866-71.

[41] Vogel J, Luisi BF. Hfq and its constellation of RNA. Nat Rev Microbiol 2011; 9: 578-89.

[42] Waters LS, Storz G. Regulatory RNAs in bacteria. Cell 2009; 136: 615-28.

[43] Kolsto AB, Tourasse NJ, Okstad OA. What sets *Bacillus anthracis* apart from other *Bacillus* species? Annu Rev Microbiol 2009; 63: 451-76.

[44] Fox, GE, Pechman, KR, Woese, CR. Comparative cataloging of 16S ribosomal ribonucleic acid: molecular approach to procaryotic systematic. Int J Syst Bacteriol 1977; 27: 44-57.

[45] Black, GE, Snyder, AP, Heroux, KS. Chemotaxonomic differentiation between the *Bacillus cereus* group and *Bacillus subtilis* by phospholipid extracts analyzed with electrospray ionization tandem mass spectrometry. J Microbiol Meth 1997; 28: 187-99.

Received: May 5, 2013 Revised: August 4, 2014 Accepted: March 30, 2015