Transition Phase Regulator AbrB Positively Regulates the sip1Ab1 Gene Expression in Bacillus thuringiensis

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ABSTRACT Bacillus thuringiensis secreted insecticidal proteins (Sip) are a secretion that is toxic to coleopteran pests. However, the transcriptional mechanism of sip genes is still unknown. The transcriptional regulation of the sip1Ab1 gene and the expression of the Sip1Ab1 protein were investigated in this study. The results demonstrated that the secretion of the Sip1Ab1 protein in HD73 was almost the same as that in the original QZL38 strain during the transition phase. Analysis of the β-galactosidase activities of sip1Ab1-lacZ in both the HD73 and abrB mutant strains indicated that the transcription of sip1Ab1 is dependent on AbrB. Electrophoretic mobility shift assays showed that AbrB could bind with the sip1Ab1 promoter, and two binding sites of AbrB in the region of the promoter of sip1Ab1 were determined by DNase I footprinting assays. All of the above-described results proved that AbrB positively regulates the sip1Ab1 gene.

IMPORTANCE Bacillus thuringiensis Sip proteins are secreted insecticidal toxins that are toxic to coleopteran pests. In this study, we investigated the transcriptional mechanism of the sip gene and showed strong evidence that Sip1Ab1 is secreted in the transition phase and that AbrB, a transition phase regulator that is usually a repressor, positively and directly regulates sip1Ab1. Reports of AbrB positive regulation are rare, even in Bacillus subtilis. To the best of our knowledge, no toxic gene has been reported to be positively regulated by AbrB in Bacillus species.

KEYWORDS AbrB, Bacillus thuringiensis, sip1Ab1, transition phase regulator

B. thuringiensis is a Gram-positive, spore-forming bacterium that can be classified in the Bacillus cereus group (1). It is characterized by the formation of parasporal crystal proteins and spores during the stationary phase of its growth cycle (2). These proteins possess highly specialized insecticidal activities against numerous insect species, including members of Lepidoptera, Coleoptera, and Diptera (3, 4). Some B. thuringiensis strains can secrete proteins during the vegetative growth phase. These secreted proteins are designated vegetative insecticidal proteins (Vip) and secreted insecticidal protein (Sip), which have insecticidal activity and extend the overall host range (5–7).

Only two kinds of Sip proteins, which are mainly secreted by vegetative cells, have been found thus far (8). Donovan first discovered the Sip1Aa1 protein (encoded by sip1Aa1) in strain ED2158 and studied its insecticidal activity against Coleoptera insects. Sip1Aa1 can cause tobacco aphids, cotton bollworm larvae, and maize root leaf beetles to shrink and lose weight (8). It has a lethal or growth-inhibiting effect on Leptinotarsa species, Diabrotica undecimpunctata howardi, and Diabrotica virgifera virgifera. Another gene, which is highly similar to sip1Aa1, was initially obtained from B. thuringiensis strain QZL38 and named sip1Ab1. Sip1Ab1 showed insecticidal activity against...
Colaphellus bowringi Baly (9). Sip1Aa1 and Sip1Ab1 both exhibit typical predicted Gram-positive consensus secretion signals at the 30th amino acid (10), and they also share 46% similarity to the 36-kDa Mtx3 mosquitocidal protein (ETX_MTX2 protein family) (8, 11). However, the regulatory mechanism of Sip protein expression is still unknown.

In Bacillus subtilis, AbrB is a global regulatory factor that regulates gene transcription in the log phase or transition phase (12). Purified AbrB protein binds specifically to fragments of DNA containing the promoters it affects (13). AbrB directly regulates more than 100 genes and influences hundreds more indirectly (14, 15). AbrB mainly functions as a transcriptional repressor for gene transcription, but it also acts as an activator (16) involved in biofilm formation, antibiotic production, capacity development, extracellular enzyme production, motility, and sporulation (17–22). Some examples of positive regulation of AbrB have been reported, such as scoC, rbs, and citB genes (16, 17, 23). Bacillus anthracis AbrB negatively regulates the toxin genes pagA, lef, and cya, which have higher transcriptional activity in the log phase (24), but the regulation is not direct (25).

The Sip1Ab1 proteins in strain QZL38 and their heterologous expression in strain HD73 were analyzed in this study. The transcription activities of the sip1Ab1 gene promoter in the HD73 wild-type strain and abrB mutant strain were analyzed using β-galactosidase. Electrophoretic mobility shift assays and DNase I footprinting assays showed the relationship between AbrB and the sip1Ab1 promoter.

**RESULTS**

Sip1Ab1 is secreted in the transition phase. In order to clarify the expression of the Sip1Ab1 protein in strain QZL38 and its heterologous expression in strain HD73, the promoter and open reading frame (ORF) of sip1Ab1 were ligated into the pHT304 vector and then electroporated into strain HD73. Time zero ($T_0$) was defined as the end of the exponential growth phase. The forespore septum was formed at 13 h after $T_0$ ($T_{13}$) (26). $T_0$ to $T_{13}$ covers the transition phase in LB medium of the HD73 strain. The same volume of the culture supernatants at $T_0$, $T_6$, $T_{12}$, and $T_{18}$ in LB medium were collected and concentrated to 2 ml using a dialysis bag. The same loading volumes of strains QZL38 (Fig. 1A) and HD73 (Fig. 1B) bacteria in the culture supernatant were subjected to SDS-PAGE analysis. Through analysis of the protein characteristics and protein molecular weight, the protein bands indicated by the arrows in the figure were identified by mass spectrometry (see Materials and Methods) and were confirmed to be the Sip1Ab1 protein. The Sip1Ab1 protein had the highest expression at $T_{13}$ in strain QZL38, and was heterologously expressed in strain HD73. This result suggested that the Sip1Ab1 protein was regulated and expressed during the transition phase. This also means that the transition phase regulator may be involved in the expression of the sip1Ab1 gene. When we selected some global regulatory factors, namely, Spo0A,
SigH, CcpA, and AbrB, and tested the regulatory relationship with sip1Ab1, only AbrB had a regulatory effect. AbrB positively regulates the sip1Ab1 gene. Strain QZL38 contains 1 chromosome and 6 plasmids in, and the sip1Ab1 gene (RS27950) is located on plasmid 2. The main cry genes include cry8Ab-like, cry8Ca1, cry8Ea1, and cry8Fa1, and their positions on plasmid 2 are shown in Fig. 2A. To determine the transcriptional start site of the sip1Ab1 gene, a 5′ rapid amplification of cDNA ends (RACE)-PCR experiment was performed (see Materials and Methods). The transcriptional start site (TSS) was confirmed to be a G located 36 nucleotides upstream of the QZL38 sip1Ab1 translational start codon (ATG) (Fig. 2B). The analysis of the QZL38 sip1Ab1 promoter region contains −35 (TAATATAA) and −10 (TTATAATTA) regions from the transcriptional start site (Fig. 2B).

To clarify the transcriptional mechanism of sip1Ab1, the HD ΔabrB strain was constructed using the principle of homologous recombination. A P_{sip1Ab1-L} lacZ fusion was constructed and transformed into the B. thuringiensis HD73 and HD ΔabrB strains. Assays of β-galactosidase activity were performed to compare the activities of cells cultured in LB medium at 30°C with shaking at 220 rpm. T₀ is the end of the exponential growth phase, and Tₙ means n hours after T₀. Each value represents the mean of at least three independent replicates. Error bars show the standard deviations.

**A** B. thuringiensis HD73. (A) Map of the cry genes and sip1Ab1 locus in Bacillus thuringiensis QZL38 plasmid 2 and construction of P_{sip1Ab1-L} (−531 to +36), P_{sip1Ab1-F} (−531 to −202) and P_{sip1Ab1-S} (−201 to +36) promoters. Bar, 1 kb. Intergenic regions are not to scale. (B) Sequence analysis of 567 bp upstream of Bacillus thuringiensis QZL38 sip1Ab1 ATG start codon. The transcriptional start site (TSS) and the putative −35 and −10 motifs are indicated with shades of gray. The two AbrB binding sites are indicated with underlines. (C) The activities of two sip1Ab1 promoters (P_{sip1Ab1-L} and P_{sip1Ab1-S}) were assessed by lacZ fusions in HD73 and HD73 ΔabrB strains. Assays of β-galactosidase activity were performed to compare the transcriptional activity of the sip1Ab1 gene promoter. In other words, AbrB positively regulates the sip1Ab1 gene.

**AbrB binds to the sip1Ab1 promoter.** To test where AbrB directly regulates the sip1Ab1 gene, AbrB-His protein was purified via nickel column affinity chromatography. The desalting AbrB protein was dissolved in 20 mM Tris-HCl (pH 8.0). A 567-bp promoter for electrophoretic mobility shift assays (EMSA), so we first selected a region of the promoter (−201 to +36) (shown in Fig. 2A), P_{sip1Ab1-S} for EMSA. 6-carboxyfluorescein (FAM)-labeled fragments containing the promoter regions were incubated with increasing concentrations of AbrB. Protein-probe binding

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**FIG 2** Transcriptional activity of the sip1Ab1 promoter in B. thuringiensis HD73. (A) Map of the cry genes and sip1Ab1 locus in Bacillus thuringiensis QZL38 plasmid 2 and construction of P_{sip1Ab1-L} (−531 to +36), P_{sip1Ab1-F} (−531 to −202) and P_{sip1Ab1-S} (−201 to +36) promoters. Bar, 1 kb. Intergenic regions are not to scale. (B) Sequence analysis of 567 bp upstream of Bacillus thuringiensis QZL38 sip1Ab1 ATG start codon. The transcriptional start site (TSS) and the putative −35 and −10 motifs are indicated with shades of gray. The two AbrB binding sites are indicated with underlines. (C) The activities of two sip1Ab1 promoters (P_{sip1Ab1-L} and P_{sip1Ab1-S}) were assessed by lacZ fusions in HD73 and HD73 ΔabrB strains. Assays of β-galactosidase activity were performed to compare the activities of cells cultured in LB medium at 30°C with shaking at 220 rpm. T₀ is the end of the exponential growth phase, and Tₙ means n hours after T₀. Each value represents the mean of at least three independent replicates. Error bars show the standard deviations.
caused slow migration. Competitive gel shift assays were performed with labeled DNA probes and approximately 200-fold unlabeled DNA targets, respectively. As shown in Fig. 3A, 200-fold unlabeled DNA could dissociate most of the AbrB from the labeled promoter probe. The data shown in the figure confirm that AbrB directly binds to \( P_{sip1Ab1-S} \) (Fig. 3A). To determine the AbrB binding site in the \( sip1Ab1 \) promoter, a DNase I footprinting assay was performed using the same promoter fragment used in the EMSA. A fragment (5'-AGAATTTCTCCTTATATAACAAT-3') of \( P_{sip1Ab1-S} \) was protected via AbrB binding (Fig. 3B), corresponding to the underlined sequence in the \( sip1Ab1 \) promoter region shown in Fig. 2B.

We compared the transcriptional activities of \( P_{sip1Ab1-S} \) and \( P_{sip1Ab1-L} \). The results showed that the activity of \( P_{sip1Ab1-S} \) was lower than that of \( P_{sip1Ab1-L} \) in wild-type HD73, and it was also significantly abolished in the HD \( \Delta abrB \) strain compared to that in the wild-type HD73 strain (Fig. 2C). We hypothesized that the promoter part \( P_{sip1Ab1-F} \) (−531 to −202) may contain an AbrB binding site. The ability of AbrB to bind to \( P_{sip1Ab1-F} \) was examined via EMSA. The data shown in Fig. 3 confirmed that AbrB directly binds to \( P_{sip1Ab1-F} \) (Fig. 3C), and the DNase I footprinting assay showed that a fragment (5'-CATATGGAAGAGAAATACCCAG-3') of \( P_{sip1Ab1-F} \) was protected via AbrB binding (Fig. 3D), corresponding to the underlined sequence in the \( sip1Ab1 \) promoter region shown in Fig. 2B. This indicates that AbrB can bind with two regions of the \( sip1Ab1 \) promoter and directly regulate the \( sip1Ab1 \) gene.

To determine the roles of two AbrB binding sites, promoter fragments from 531 to +36 from which binding site 1 or binding site 2 was deleted were fused to \( lacZ \) (Fig. 4A). The \( \beta \)-galactosidase activity assays (Fig. 4B) indicated that the transcriptional activity of \( P_{sip \_ delete1} \) in wild-type HD73 is no different from that of \( P_{sip1Ab1-S} \). The transcriptional activity of \( P_{sip \_ delete2} \) was very low. The transcriptional activity of \( P_{sip \_ delete1} \) was
significantly abolished in the HD ΔabrB strain compared to that in the wild-type HD73 strain.

**DISCUSSION**

Sip is a secreted protein, and Sip1Ab1 was secreted during the transition period (Fig. 1). Sporulation medium lacking nutrients has also been used to produce the Sip1Ab1 protein, but production was extremely low (data not shown). The Sip1Ab1 protein was secreted in sufficient nutrient medium (Fig. 1). Thus, nutrient-poor medium is not suitable for Sip1Ab1 protein expression, and medium that can extend the transition phase is suitable for Sip1Ab1 expression.

In this study, AbrB positively regulated the toxin gene in *B. thuringiensis* (Fig. 2C). AbrB mostly negatively regulates gene transcription, and it is considered to be a repressor of gene expression, although several genes were positively regulated by AbrB in *B. subtilis*. All previous studies showed that AbrB negatively regulates gene expression in the *B. cereus* group. In *B. cereus*, overexpression of AbrB resulted in a nontoxin phenotype, and the Spo0A-AbrB circuit negatively regulated *ces* and repressed cereulide production (27). In *B. anthracis*, AbrB negatively regulates toxin genes (24). In *B. thuringiensis*, AbrB repressed biofilm formation and motility, which showed similarities to those in *B. subtilis* (28). AbrB also negatively regulated the immune inhibitor metalloprotease *inhA1* (29). Currently, no toxin gene has been reported to be positively regulated by AbrB.

AbrB binds two regions of the sip1Ab1 promoter, and the binding sequences were confirmed here (Fig. 3). P_{sip1Ab1-L} and P_{sip1Ab1-S} each have a binding sites, and the difference in activity indicates that the two sites have distinct roles. A previous study reported that AbrB bound to the atxA promoter in *B. anthracis*, and the binding sequence was identified (25). Since both *B. thuringiensis* and *B. anthracis* belong to the *B. cereus* group, the homology of AbrB between them is 99% (see Fig. S1 in the supplemental material). Thus, we performed an alignment of the AbrB binding sequences of...
MATERIALS AND METHODS

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TABLE 1 Strains and plasmids used in this study

| Strain or plasmid | Characterization* | Source and/or reference |
|-------------------|-------------------|-------------------------|
| **Strains**       |                   |                         |
| *Bacillus thuringiensis* |                   |                         |
| HD73              | *B. thuringiensis* subsp. kurstaki, carrying cry1Ac gene | Lab stock |
| HD (ΔabrB)        | *B. thuringiensis* HD73 abrB gene insertion mutant; Kan' | This study |
| HD (sip1Ab1)      | HD73 strain containing plasmid pHTSip1Ab1 | This study |
| HD (P<sub>ap</sub>1-4) | HD73 strain containing plasmid pHTP<sub>ap</sub>4-1 | This study |
| HD (P<sub>ap</sub>1-4-F) | HD73 strain containing plasmid pHTP<sub>ap</sub>4-1-F | This study |
| HD (P<sub>ap</sub>1-4-L) | HD73 strain containing plasmid pHTP<sub>ap</sub>4-1-L | This study |
| HD (P<sub>ap</sub>1-4-L) | HD73 strain containing plasmid pHTP<sub>ap</sub>4-1-L | This study |
| HD (P<sub>ap</sub>1-4 delete1) | HD73 strain containing plasmid pHTP<sub>ap</sub>1-4 delete1 | This study |
| HD (P<sub>ap</sub>1-4 delete2) | HD73 strain containing plasmid pHTP<sub>ap</sub>1-4 delete2 | This study |
| HD ΔabrB (P<sub>ap</sub>1-4-1) | HD (ΔabrB) strain containing plasmid pHTP<sub>ap</sub>4-1 | This study |
| HD ΔabrB (P<sub>ap</sub>1-4-1) | HD (ΔabrB) strain containing plasmid pHTP<sub>ap</sub>4-1 | This study |
| HD ΔabrB (P<sub>ap</sub>1-4 delete1) | HD (ΔabrB) strain containing plasmid pHTP<sub>ap</sub>1-4 delete1 | This study |
| HD ΔabrB (P<sub>ap</sub>1-4 delete2) | HD (ΔabrB) strain containing plasmid pHTP<sub>ap</sub>1-4 delete2 | This study |
| QZL38            | *B. thuringiensis* carrying cry8 genes and sip1Ab1 gene | Lab stock |
| E. coli ET      | F' dam-13::Tn9 dcm-6 hsdM hsdR recF143 zji-202::Tn10 galK2 gatT22 ara 14 pacF1 xyl-5 leuB6 thi-1; for generation of unmethylated DNA | Lab stock |

| **Escherichia coli** |                   |                         |
| BL21              | *Escherichia coli* | Lab stock |
| BL21 (pETabrB)    | BL21 strain containing plasmid pETabrB | This study |
| BL21 (pET)        | BL21 strain carrying pET21b | Lab stock |

| **Plasmids**       |                   |                         |
| pMAD              | Amp', Erm' shuttle vector; thermosensitive origin of replication | Lab stock (40) |
| pMADΔabrB         | pMAD with abrB insertion fragment | This study |
| pHT304            | Amp', Erm'; *E. coli*-B. thuringiensis shuttle | Lab stock |
| pHT315            | Amp' Erm'; *E. coli*-B. thuringiensis shuttle | Lab stock |
| pET21b            | Expression vector; Amp'; 5.4 kb | Lab stock |
| pETabrB           | pET21b containing abrB gene; Amp' | This study |
| pHTP<sub>ap</sub>4-1 | pHT304-18Z carrying the promoter of sip1Ab1 | This study |
| pHTP<sub>ap</sub>4-1-F | pHT304-18Z carrying half of the promoter of sip1Ab1 | This study |
| pHTP<sub>ap</sub>4-1-L | pHT304-18Z carrying half of the promoter of sip1Ab1 | This study |
| pHTSip1Ab1        | pHT304 carrying sip1Ab1 and P<sub>ap</sub>4-1 | This study |
| pHTP<sub>ap</sub>1-4 delete1 | pHT304-18Z carrying the promoter of sip1Ab1 with binding site 1 deleted | This study |
| pHTP<sub>ap</sub>1-4 delete2 | pHT304-18Z carrying the promoter of sip1Ab1 with binding site 2 deleted | This study |
| pMAD19-T<sub>ap</sub>1-4-F | pMAD19-T carrying half of the promoter of sip1Ab1 | This study |
| pMAD19-T<sub>ap</sub>1-4-L | pMAD19-T carrying half of the promoter of sip1Ab1 | This study |

*Bivalent, kanamycin resistance; Amp', ampicillin resistance; Erm', erythromycin resistance.*

*B. thuringiensis* and *B. anthracis*. A conserved DNA sequence that was analyzed by MEME, TGGTWAAAARGGAA, was identified (see Fig. S2 in the supplemental material). The conserved sequence is not consistent with the AbrB consensus binding sequence, which in *B. subtilis* consists of bipartite TGGNA motifs separated by 4 to 5 bp (30), but they were all extremely AT rich. As Koehler mentioned, the *B. anthracis* and *B. subtilis* AbrB binding sites are also somewhat different (31). Via AbrB protein sequence alignment analysis, it was found that *B. anthracis* and *B. thuringiensis* AbrB proteins are 85% identical to *B. subtilis* AbrB (Fig. S1), and the last 32 residues are significantly different (24). AbrB probably recognizes a three-dimensional DNA structure rather than a typical DNA sequence (13, 29, 32, 33). Here, we suggest that the AbrB consensus binding sequence in the *B. cereus* group is different from that in *B. subtilis*. Our results provide further understanding of the AbrB binding sequence and its regulatory mechanism.

**MATERIALS AND METHODS**

**Strains, plasmids, and growth conditions.** The strains and plasmids used in this study are summarized in Table 1. *Escherichia coli* strains DH5α and BL21 were used as hosts for molecular cloning and protein expression, respectively. *E. coli* SC110 (also called ET12567) was used for transformation into *B. thuringiensis*, as described previously (34). *B. thuringiensis* HD73 was used as the recipient strain to monitor gene transcriptional activity and manipulate the gene cloning of *B. thuringiensis* (35, 36). HD73 and its derivatives were routinely grown at 30°C in LB broth or on LB agar plates supplemented with either erythromycin (5 μg/ml) or kanamycin (100 μg/ml) when required.
**TABLE 2 Sequences of oligonucleotide primers used in this study**

| Primer name       | Oligonucleotide sequence (′5′-′3′) (restriction enzyme)* |
|-------------------|--------------------------------------------------------|
| P_{sip1Ab1-L}-F   | AACACGACAGCTTTAATGATAGGGA (PstI)                       |
| P_{sip1Ab1-L}-R   | CGGATCCAAATATGCTCAGTACC (BamHI)                       |
| P_{sip1Ab1-F}-F   | AACACGACGATCTTAAAGGACATAGGGAATGAGG (PstI)             |
| P_{sip1Ab1-F}-R   | CGGATCCCTTAAATGCTCAGTACCC (BamHI)                     |
| sip1Ab1-F         | AACACGACGATCATAATCCATATGAGGGAATGAGG (PstI)            |
| sip1Ab1-R         | CGGATCCCTTAAATCCATATGAGGGAATGAGG (PstI)               |
| 304-F             | CTATGACCATGATTTACGCAAGCTTGCAGTC                          |
| 304-R             | GGATGTGCTGCAAGGCGATTAAGTTGG                              |
| 304-18Z F         | CGTAACTCTAGTCAATTTCCATACCC (5′ FAM labeled)            |
| 304-18Z R         | CGCCAGGGTTTTCCAGTACCC (FAM labeled)                    |
| abbrB-a           | TACATGCTATGCTGATTCAATCGGACATATGCATGCTGTAATTAT           |
| abbrB-b           | CCTAAATGTTGCCTGTTTTGATTGCTC (ATTTTCC)                  |
| Km-F              | CCAAAATGCAGAATCTAAAAAACGCGAACATTGGAG                  |
| Km-R              | GTCTATTTTTCGTGTTGATATAATATCTCTTGCTC (CGTGTAG)         |
| abbr-c            | GGCCTACGAGGAATTTTATATCGAAACAGCAAAATACCC (BamHI)       |
| abbr-d            | TCGACCGGTCGTCGAGAACATTACCC (SalI)                     |
| AbrB-F            | CGGATCCGATATTCAATCTACTCTGTTAATGAGTACGCACTCC (BamHI)   |
| AbrB-R            | GGCTAACCTTTCTCTGTTCGATATAATTCTACCGGCACTG               |
| JDWΔabrB-F        | GCCATGACGAAAGCTTCTCAGTCC (PstI)                       |
| JDWΔabrB-R        | GGATAGCTGCTGATGAGTGATATGACCC (BamHI)                  |
| sip1Ab15′-race-R  | TTCTTTAATATGCCAATCTATGGTTTCTTTCC (5′ FAM labeled)     |
| P_{sip1F}-F       | CATCCGATAAAGGAACAGATGAATT                           |
| P_{sip1F}-R       | AATTATCCCTCCTCATCTTCTTTTG                            |
| P_{sip1F-R}       | CAGCTATAACATATGTTGGAAGAAA                              |
| P_{sip1-R}        | TTAAAAAGTGACCTTTCTTAAAAATCC                          |
| M13F-FAM          | GTAAAGCAGCGGCAGT (5′ FAM labeled)                    |
| M13R-FAM          | CAGCGAACGAGCTATG (FAM labeled)                       |

*FAM, 6-carboxyfluorescein. The restriction sites are underlined and bolded.

**Secretion of Sip1Ab1 in QZL38 and HD73 strains.** A single *B. thuringiensis* colony was inoculated in 5 ml LB medium and grown at 30°C with shaking overnight, and then 1 ml of the bacterial solution was added to 100 ml of LB medium and incubated to T₀ (T₀ is the end of the exponential growth phase, and T₀ is n hours after the end of the exponential growth phase, T₀, T₀, and T₀). A 50-ml aliquot of bacterial solution was collected and centrifuged (4°C at 9,000 rpm for 10 min), and then the supernatants were collected into the dialysis bag in each period. An appropriate amount of polyethylene glycol (PEG) 8000 was also needed outside the dialysis bag. The concentration time was generally 6 to 10 h, and the temperature was 4°C. The proteins were dissolved in 2 ml of 20 mM Tris-HCl, and the same volume was centrifuged (13,000 rpm for 10 min), and then the pellets were stored at −20°C until use. The α-Galactosidase activity analysis was conducted as previously described (37) and are expressed as Miller units. The reported values represent the averages from at least three independent assays.

**β-Galactosidase activity analysis.** The *B. thuringiensis* strains were cultivated in LB medium with shaking (220 rpm at 30°C). Aliquots (2 ml) of cultures were collected every 2 h during the T₀ to T₀, T₀, and T₀ intervals (T₀ indicates the end of the exponential growth phase, and T₀ indicates n hours after T₀). The cells were centrifuged (13,000 × g for 1 min), and the pellets were stored at −20°C until use. β-Galactosidase activities were measured as previously described (37) and are expressed as Miller units. The reported values represent the averages from at least three independent assays.

**RACE analysis.** The total RNA was extracted from the QZL38 DNA in LB until the T₀ stage, and reverse transcription-PCR was conducted as previously described (38). We used the SMARTer RACE (switching mechanism at the 5′ end of the RNA transcript-rapid amplification of cDNA ends) cDNA amplification kit (Clontech, Mountain View, CA) to determine the transcription start site, following the manufacturer's instructions. A 1598-bp fragment, containing the 567 bp upstream of the QZL38 sip1Ab1 ATG start codon and the sip1Ab1 open reading frame, was amplified and ligated to the linearized pHT304 plasmid. The recombinant plasmids were introduced into E. coli DH5α. The resulting strains were placed on agar plates supplemented with erythromycin and verified by PCR.

**Construction of the pHTspIp1Ab1 and pHTP\_up\_lacZ expression vectors.** The promoter sequences of the sip1Ab1 gene were amplified from the QZL38 genomic DNA (GenBank accession no. CP032609; region, 192552 to 193646) using different primers (Table 2). A 567-bp fragment located from −531 to +36 was PCR amplified from strain QZL38 with primers Psip1Ab1-L-F and Psip1Ab1-L-R, a 237-bp fragment located from −201 to +36 was PCR amplified from strain QZL38 with primers Psip1Ab1-S-F and Psip1Ab1-S-R, a 329-bp fragment located from −531 to −202 was PCR amplified from strain QZL38 with primers Psip1Ab1-L-F and Psip1Ab1-L-R, and a 533-bp/554-bp fragment which contained −531 to +36 except for binding site 1/binding site 2 was synthesized (Sangon Biotech, Shanghai) and ligated to the linearized pHT304 plasmid, which contains a promoterless lacZ gene. A 1598-bp fragment, containing the 567 bp upstream of the QZL38 sip1Ab1 ATG start codon and the sip1Ab1 open reading frame, was amplified and ligated to the linearized pHT304 plasmid. The resulting strains were placed on agar plates supplemented with erythromycin and verified by PCR.
codon (ATG), was designed as the specific reverse primer. NestRace was the forward primer provided in the kit (Clontech, Mountain View, CA). sip1Ab15’race-R and NestRace were used as specific primers for amplifying the 5’ end of Psip, cDNA. The sequences of the primers used in this study are shown in Table 2.

Screening of HD ΔabrB mutants. The HD73 abrB mutant was constructed using the principle of homologous recombination. The method is briefly described as follows. A 709-bp upstream region containing a 15-bp overlap with the 5’ end of abrB (abrB fragment A) and a 729-bp downstream region containing a 21-bp overlap with the 3’ end of abrB (abrB fragment B) were amplified from B. thuringiensis HD73 genomic DNA with the abrB-a/abrB-b and abrB-c/abrB-d primer sets, respectively. The kanamycin resistance gene (kan, 1,473 bp) was amplified with the Km-F/Km-R primer set using the ΔsigH mutant as a template. Subsequently, a long flanking PCR was performed with abrB fragment A, the kan fragment, and abrB fragment B as the templates, in that order, and the abrB-a/abrB-d primer set to generate a long fragment (2,828 bp). The resulting DNA fragment was doubly digested with BamHI and SalI and cloned into the erythromycin (ERY)-resistant, temperature-sensitive suicide plasmid pMAD. The recombinant plasmid was named pMADΔabrB. The recombinant plasmid was transferred to E. coli ET for demethylation and then electroporated into strain HD73. The strain was subjected to high-temperature mutation at 37°C, and a strain named HD (pMADΔabrB), with kanamycin resistance and no erythromycin resistance, was selected. Using the mutant cassette outer primers JDwabrB-1 and JDwabrB-2, the wild-type strain HD73 and the kanamycin-resistant and ERY-resistant strain were used as the templates to identify the mutant strain, and the obtained mutant strain was named HD ΔabrB.

Purification of the AbrB protein. The BL21 (pETabrB) strain was cultured in LB medium containing 100 μg/ml ampicillin at 37°C and 220 r/min to an optical density at 600 nm (OD600) of 0.7 to 1.0 and then added to a final concentration of 0.5 mmol/liter isopropyl-β-D-thiogalactopyranoside (IPTG) induced at 18°C and 150 rpm for 12 h. The cells were collected by centrifugation at 9,000 rpm for 10 min at 4°C. The cells were suspended in 50 mM Tris-HCl (pH 8.0). The suspension was ultrasonically disrupted on ice for 6 min (CP750, ultrasonic power 70%, ultrasound 3 s, and pause 5 s; Cole-Parmer). The supernatant and precipitate were separated by a low-temperature centrifuge (12,000 rpm for 10 min). The supernatant containing soluble AbrB protein was placed in a well-balanced nickel affinity chromatography column, and the His-tagged AbrB protein was fully combined with the column. Then, the protein was washed with 10 column volumes of equilibration buffer (20 mM/liter Tris-HCl (pH 8.0), 0.5 mM/liter NaCl, and 20 mM/liter imidazole), and finally the proteins eluted by the 10 ml of elution buffer (20 mM/liter Tris-HCl (pH 8.0), 0.5 M/liter NaCl, and 250 mM/liter imidazole) were collected. SDS-PAGE was used to detect the eluted protein samples. The purified protein samples were desalted using the Äkta protein purifier, and the desalted protein was dissolved in 20 mM Tris-HCl (pH 8.0).

Electrophoretic mobility shift assays. The chosen gene of promoter sequences was amplified from QZL38 genomic DNA using different primers labeled with 6-carboxyfluorescein (FAM) (Table 2). The gel retardation assay determines the binding of the DNA fragment to the protein; 20 μl of the reaction system contains labeled DNA, different concentrations of AbrB protein and binding buffer [10 mM/liter Tris-HCl, 0.5 mM/liter dithiothreitol (DTT), 50 mM/liter NaCl, 500 ng poly(dI:dC) (pH 7.5), and 4% (vol/vol) glycerol]. The reaction was performed at 25°C for 30 min. The reaction product was detected via electrophoresis in 8% (wt/vol) non-denaturing polyacrylamide gel in TBE buffer (90 mM/liter Tris-base, 90 mM/liter boric acid, and 2 mmol/liter EDTA [pH 8.0]) [Mini-Protean system, 160 V, 4°C, 1 h; Bio-Rad]. The non-denatured gel was scanned with a fluorescent gel imaging system (FLA Imager FLA-5100; laser, 473 nm; voltage, 900 V; filter, 526-000/01; Fujiﬁlm).

DNase I footprinting assay. The promoter region was PCR amplified with 2× high-fidelity DNA polymerase premix (Tolo Biotech, Shanghai, China) from the plasmids pMAD19-TPsip and pMAD19-TPsipΔsip1 using M13F (FAM) and M13R primers to prepare the fluorescent FAM-labeled probes. The FAM-labeled probes were purified using the Wizard 5V gel and PCR clean-up system (Promega, USA) and were quantified with a NanoDrop 2000C instrument (Thermo, USA). The DNase I footprinting assays were performed following Wang et al. (39).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, DOCX file, 0.2 MB.

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We declare no conflicts of interest.

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