AEM
Journals.ASM.org

Identification of the Promoter in the Intergenic Region between *orf1* and *cry8Ea1* Controlled by Sigma H Factor

Lixin Du,[a,b] Lili Qiu,[a] Qi Peng,[a] Didier Lereclus,[c] Jie Zhang,[a] Fuping Song,[a] and Dafang Huang[d]

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China[a]; Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences, IPM Centre of Hebei Province, Baoding, China[b]; INRA, UMR1319 Micalis, Génétique Microbienne et Environnement, La Minière, Guyancourt, France[c]; and Biotechnology Research Institute, Chinese Academy of Agricultural Science, Beijing, China[d]

Bacillus thuringiensis **Cry8Ea toxin is specifically toxic to larvae of the Asian cockchafer, *Holotrichia parallela*. Here we investigated the mechanism of transcriptional regulation of the *cry8Ea1* gene. Reverse transcription-PCR (RT-PCR) results indicated that *cry8Ea1* and an upstream gene (*orf1*) were cotranscribed. Transcriptional fusions with the *lacZ* gene demonstrated that transcription of the *cry8Ea1* gene started from two promoters: P_{orf1}, which is located upstream of the *orf1* gene, and P_{cry8E}, located in the intergenic region mapping between *orf1* and *cry8Ea1*. Of the known, similar *orf1-cry* operons, this is the first report of the existence of a promoter in the intergenic region between the *orf1* and *cry* genes. The transcriptional activity of P_{orf1} was found during sporulation in *B. thuringiensis* subsp. *kurstaki* HD-73 and was almost abolished in the *sigE* mutant, while the transcriptional activity of P_{cry8E} was detected after the end of the exponential phase in HD-73 and was considerably lower in the *sigH* mutant. The transcription start sites generated by the two *cry8Ea1* promoters were determined by the 5′ -SMARTer rapid amplification of cDNA ends (RACE) method. The −35 and −10 regions of P_{orf1} and P_{cry8E} showed high sequence similarity with the σ^E and σ^H promoters, respectively. These results indicated that P_{orf1} is controlled by the σ^E factor and P_{cry8E} by the σ^H factor.**

Bacillus thuringiensis is a Gram-positive, spore-forming bacterium that produces parasporal crystal proteins. These parasporal crystal proteins are encoded by *cry* genes and possess a highly specific insecticidal activity against a great number of insect species. To date, more than 500 *cry* genes have been discovered (http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/). Most *B. thuringiensis cry* genes have a monocistronic structure, with the exceptions of *cry2Aa* (25), *cry2Ac* (27), *cry9Ca* (15), *cry9Ec* (23), *cry11A* (11), and *cry15A* (8), which form operons with at least one upstream open reading frame (ORF), named *orf1* or *p19*. However, no promoter activity in the intergenic region between the *orf1* and *cry* genes was reported in the previous studies.

Transcription of *cry1A* depends on the BtI promoter in the early stage of the stationary phase and on the BtII promoter in the late stage of the stationary phase (26). They are regulated by sporulation-specific sigma factors, σ^E and σ^K, respectively (6, 9, 10). Most of the known crystal protein genes (3, 5) contain either BtI alone or both BtI and BtII. Among these, some *cry* genes (i.e., *cry1Ac* and *cry4A*) with two or more promoters are weakly controlled by σ^H during the transition phase and strongly controlled by sporulation-specific sigma factors (18, 28). In contrast, *cry3A* expression is not dependent on sporulation-specific sigma factors. The *cry3A* promoter is similar to promoters recognized by the main sigma factor of vegetative cells, σ^A (1, 2).

B. thuringiensis strain BT185 produces spheric inclusions that exhibit specific toxicity against larvae of the Asian cockchafer, *Holotrichia parallela* (29). Shu et al. cloned two homologous 130-kDa insecticidal crystal protein genes, designated *cry8Ea1* and *cry8Fa1*. Cry8Ea1 toxin is toxic to *H. parallela*, whereas no target insect was found to be susceptible to the Cry8Fa1 protein (21). The three-dimensional structure of activated Cry8Ea1 toxin was determined by X-ray crystallographic methods (14). However, the regulation of *cry8Ea1* gene expression still remains unknown. For this study, we report that *cry8Ea1* is transcribed from two promoters: one is located upstream of the *orf1* gene, and the second maps in the intergenic region between *orf1* and *cry8Ea1*. Transcription of the *cry8Ea1* gene is controlled by σ^E and σ^H.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table S1 in the supplemental material. *Escherichia coli* JM110 was used for the cloning experiments, while SCS110 was used to produce nonmethylated plasmid DNA for *B. thuringiensis* transformations (22). *B. thuringiensis* subsp. *kurstaki* HD-73 was used as a recipient strain to monitor promoter activity (12).

E. coli was cultured at 37°C in Luria-Bertani medium (1% NaCl, 1% tryptone, 0.5% yeast extract). *B. thuringiensis* strains were grown at 30°C in Schaeffer's sporulation medium (SSM) (20). Ampicillin (100 μg/ml) or erythromycin (50 μg/ml) was added to the media, when appropriate, for selection of antibiotic-resistant strains of *E. coli* and *B. thuringiensis*.

DNA manipulation and transformation. Chromosomal DNA was extracted from *B. thuringiensis* as described previously (16). Plasmid DNA was extracted from *E. coli* by the standard alkaline lysis procedure. Restriction enzymes and T4 DNA ligase were purchased from TaKaRa Biotechnology Corporation (Dalian, China). PCR product purification kits were purchased from Axygen Inc. (Hangzhou, China). *Taq* DNA polymerase and KOD DNA polymerase were purchased from New England BioLabs Ltd. (Beijing, China). All oligonucleotide primers used in this study are listed in Table S2 in the supplemental material. Standard procedures were used for *E. coli* transformation. *B. thuringiensis* cells were transformed by electroporation as previously described by Lereclus et al. (16).

Received 27 February 2012 Accepted 6 April 2012
Published ahead of print 13 April 2012
Address correspondence to Fuping Song, fpsong@ippcaas.cn, or Dafang Huang, dfhuang@mail.caas.net.cn.
Supplemental material for this article may be found at http://aem.asm.org/.

doi:10.1128/AEM.00622-12

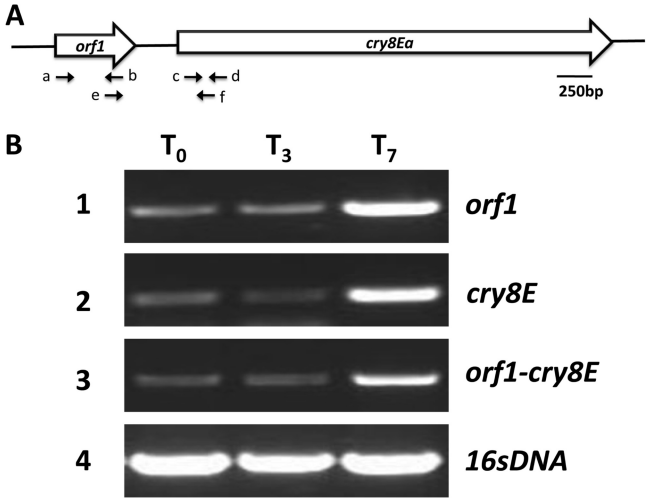

FIG 1 Analysis of *cry8Ea* operon in *B. thuringiensis* BT185. (A) *cry8E* gene locus. The letters below summarize the main primers used for RT-PCR. Primers a (RTorf1-5), b (RTorf1-3), c (RTcry8E5), d (RTcry8E3), e (RTCO), and f (RTCO) were used for RT-PCR analysis of the *orf1* and *cry8E* genes (see Table S2 in the supplemental material). (B) RT-PCR for the transcription linkages of the *orf1* and *cry8E* genes. The RNA samples were prepared at different time points of bacterial growth in SSM, as indicated. Rows 1 to 3 denote the RT-PCR products of *orf1*, *cry8E*, and *orf1-cry8E*, and the 16S rRNA gene (row 4) was used as a positive control.

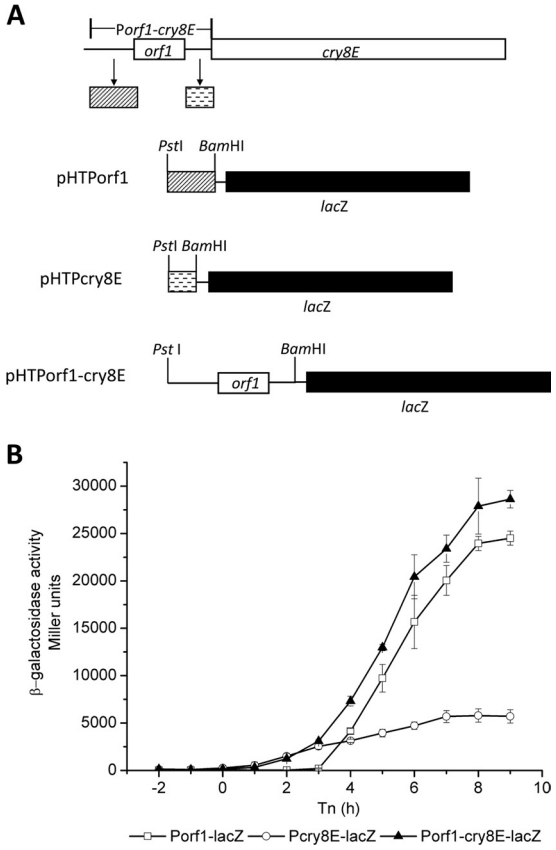

FIG 2 Characterization of the promoter region of the *cry8Ea* operon. (A) Construction of promoter fusions with the *lacZ* gene. The upstream regions of the *orf1* gene were amplified by PCR using the primers Porf1-5 and Porf1-3. The intergenic region between *orf1* and *cry8Ea* was amplified using the primers Pcry8E5 and Pcry8E3. The complete upstream region, including the upstream region of the *orf1* gene, the *orf1*gene, and the region between the *orf1* and *cry8E* genes, was amplified using the primers Porf1-5 and Pcry8E3. The three amplified fragments were inserted into pHT304-18Z to generate the transcriptional fusion plasmids pHTPorf1, pHTPcry8E, and pHTPorf1-cry8E. (B) Transcriptional analysis of the promoter region of the *cry8Ea* operon. The promoter-directed β-galactosidase synthesis of three clones was determined at the indicated times after growing the cells in SSM at 30°C. Each value represents the mean for at least three independent replicates.

Construction of *sigE* and *sigH* deletion mutant. The DNA fragments corresponding to the downstream and upstream regions of the *sigH* gene were amplified by PCR, using chromosomal DNA from HD-73 as the template and sigH1/sigH2 and sigH3/sigH4 as primers. The corresponding DNA fragments were fused by overlapping PCR, using sigH1 and sigH4 as the primers. The PCR products were then digested with BamHI-SalI restriction enzymes. The digested fragments were purified and ligated with the temperature-sensitive suicide plasmid pMAD (4), which was also treated with BamHI-SalI, to generate the recombinant plasmid pMADΩsigH. The recombinant plasmid was electroporated into the HD-73 strain. In-frame deletion of the *sigH* gene in *B. thuringiensis* was conducted following a modification of a previously described procedure (4). One verified transformant of this deletion mutant was cultured at 40°C. Colonies with no erythromycin resistance were selected, and one mutant strain, HDΔsigH, was verified by PCR identification using the primers YZsigH5 and YZsigH3, mapping outside the deletion frame, and DNA sequencing was performed.

The primers sigE1/sigE2 and sigE3/sigE4 were used to construct *sigE* deletion mutation cassettes. This fragment was integrated into pMAD to give pMADΩsigE. The deletion mutant, HDΔsigE, was selected and confirmed by PCR and Southern blotting (24).

RNA extraction and reverse transcription-PCR (RT-PCR) analyses. Total RNA was extracted from *B. thuringiensis* BT185 cells grown in SSM at stages T_0, T_3, and T_7 (T_0 is the end of the exponential phase; T_n is *n* hours after the end of the exponential phase). The bacterial cells were harvested from 1 ml of culture by centrifugation in 2-ml tubes (13,000 × *g*, 30 s, 4°C), and the cell pellets were immediately resuspended in 1 ml cold TRI Reagent (Invitrogen, San Diego, CA). The RNA was extracted with the Qiagen Easy RNA kit according to the manufacturer's instructions. The residual DNA was removed using RNase-free DNase I(New England BioLabs), and the resulting RNA samples were stored at −70°C. cDNA was synthesized from 0.5-μg aliquots of total RNA using the PrimeScript II 1st-strand cDNA synthesis kit (TaKaRa, Dalian, China) with the Random 6mer primers according to the manufacturer's instructions. The following primers were used to detect expression of the *orf1* gene and *cry8Ea1* gene: *orf1*, RTorf1-5/RTorf1-3; *cry8Ea1*, RT8E5/RT8E3; and 16S rRNA genes, 16SrDNA5/16SrDNA3. Conditions for amplification were as follows: one incubation for 5 min at 94°C, followed by 30 cycles for 1 min at 94°C for denaturation, 1 min at 54°C for annealing, and 2 min at 72°C for extension, with an extra extension at 72°C for 10 min. The amplification products were separated on 1.5% agarose gels. Negative control samples were subjected to amplification.

Determination of transcriptional start sites. To determine the transcriptional start sites, we employed the technique switching mechanism at the 5′ end of the RNA transcript-rapid amplification of cDNA ends (SMARTer RACE) cDNA amplification kit (Clontech, Mountain View, CA), following the manufacturer's instruction. Gene-specific primers, 8ERace and NestRace, were used to amplify the 5′ end of *cry8Ea* mRNA.

Construction of P_{orf1} and P_{cry8E} promoter fusions with the *lacZ* gene. The first putative promoter fragment of P_{orf1} (559 bp), which is located upstream of the *orf1* gene, was cloned from *B. thuringiensis* BT185 genomic DNA using the specific primers Porf1-5 (with a PstI restriction site) and Porf1-3 (with a BamHI restriction site). The PstI-BamHI fragment of the P_{orf1} promoter was then integrated into the vector pHT304-18Z, harboring a promoterless *lacZ* gene. The resulting plasmid, pHT-

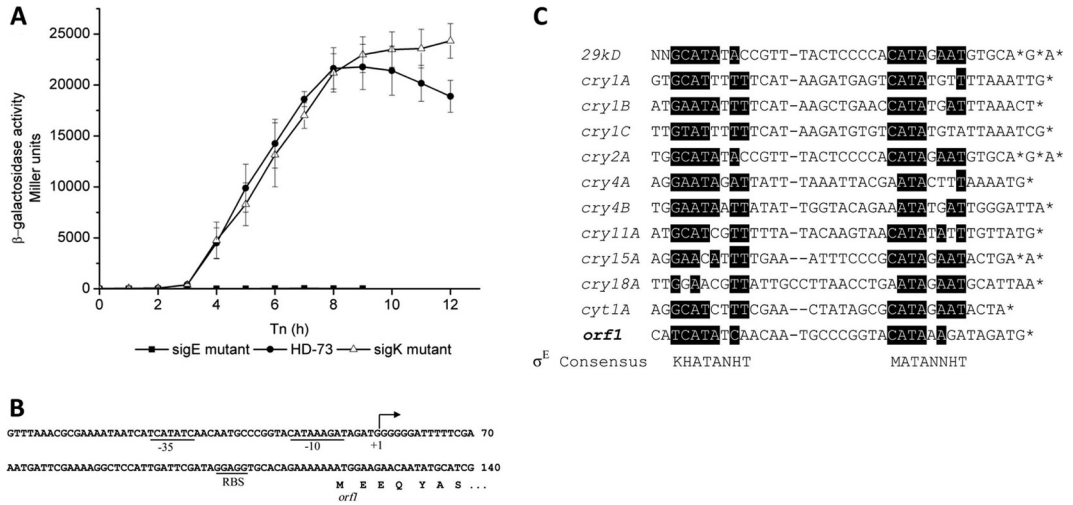

FIG 3 (A) Analysis of P_{orf1} promoter activities in the HD-73 wild-type strain (●), the *sigE* mutant (■), and the *sigK* mutant (△). The promoter-directed synthesis of β-galactosidase in the strains was determined at the indicated times after growing the cells in SSM at 30°C. Each value represents the mean for at least three independent replicates. (B) The promoter region of the operon. Transcriptional initiation (+1) is marked. The putative promoter region of the operon (−35 and −10) and the putative ribosome binding site of *orf1* (RBS) are underlined and marked. "*orf1*" below the arrow indicates the 5′-end of *orf1* and the deduced amino acid sequence. (C) Sequence similarity among the *cry* gene promoters and the consensus sequences obtained by aligning the σ^E-dependent promoters of *B. thuringiensis*. "*" indicates the transcriptional initiation sites. Consensus sequences of σ^E-dependent promoters are presented above and below the alignment, respectively. D is A, G, or T; H is A, C, or T; K is G or T; M is A or C; N is A, C, G, or T; V is A, G, or C.

Porf1, was introduced into HD-73, the *sigE* mutant, and the *sigK* mutant (13). The corresponding strains, HD(P_{orf1}-lacZ), HDΔsigE(P_{orf1}-lacZ), and HDΔsigK(P_{orf1}-lacZ), were selected by erythromycin resistance and PCR identification. Similarly, the primers Pcry8E5 and Pcry8E3 were used to construct the second putative promoter fragment, P_{cry8E} (280 bp), which was inserted into the pHT304-18Z vector to generate pHTPcry8E. The recombinant plasmid pHTPcry8E was electroporated into HD-73 and the *sigH* mutant to give HD(P_{cry8E}-lacZ) and HDΔsigH(P_{cry8E}-lacZ), respectively.

Primers Porf1-5 and Pcry8E3 were used for PCR amplification of the 1,352-bp fragment containing the upstream region of *orf1* gene and the region between the *orf1* and *cry8Ea1* genes. After digestion with PstI-BamHI, the PCR product was integrated into pHT304-18Z to generate pHTPorf1-cry8E. The resulting plasmid, pHTPorf1-cry8E, was introduced into HD-73 to generate HD($P_{orf1\text{-}cry8E}$-lacZ).

β-Galactosidase assay. *B. thuringiensis* strains containing *lacZ* fusions were grown in SSM at 220 rpm and 30°C, supplemented with appropriate antibiotics. Samples of 2 ml were taken at T_{-2} and at 1-h intervals until T_9. The cells were harvested, and the specific β-galactosidase activity of the samples was measured as previously described and expressed as Miller units per milligram of protein (17). The values reported are the means for at least three independent experiments.

RESULTS AND DISCUSSION

Sequence analysis and RT-PCR. The sequence analysis showed that *orf1* was located 286 bp immediately upstream of *cry8Ea1* (GenBank accession number AY329081) and that no potential transcriptional terminator was present between the two genes. This finding suggested that the *orf1* and *cry8Ea1* genes may form one operon. To test this hypothesis, we analyzed the transcription of the two genes. Three pairs of primers were designed according to the *orf1* and *cry8Ea1* gene sequence to detect their transcription (Fig. 1A; see also Table S2 in the supplemental material). RT-PCR was carried out with total RNA extracted from *B. thuringiensis* strain 185 at T_0, T_3, and T_7 from cultures grown in SSM (Fig. 1B). The data suggest that the *orf1* and *cry8Ea1* genes are transcribed together as one transcription unit. This coincided with the *cry2A*, *cry11A*, *cry15A*, and *cry18A* operons (8, 11, 25, 30).

Transcriptional activity of promoters from *cry8Ea* operon. The promoter region(s) of the *cry8Ea* operon was analyzed by constructing transcriptional fusions with the *lacZ* gene (Fig. 2A). The HD-73 strains containing pHTPorf1, pHTPcry8E, and pHTPorf1-cry8E were cultured in SSM, and β-galactosidase activities of the strains were assayed (Fig. 2B). The β-galactosidase activities of HD(P_{cry8E}-lacZ) and HD($P_{orf1\text{-}cry8E}$-lacZ) were detected at the onset of sporulation (T_0), while that of HD(P_{orf1}-lacZ) was detected only 3 h later (T_3). The activity of the P_{cry8E} promoter was approximately 5-fold lower than that of the P_{orf1} promoter after T_3. The higher transcriptional activity obtained with the complete *cry8Ea1* gene promoter, $P_{orf1\text{-}cry8E}$, is likely due to the combined activities of P_{orf1} and P_{cry8E}. These results suggest that *cry8Ea1* has two promoters, P_{orf1}, which has a high level of activity during sporulation, and P_{cry8E}, which is active at the onset of sporulation. The first promoter, P_{orf1}, is located upstream of the *orf1* gene. The second promoter, P_{cry8E}, is located in the intergenic region between the *orf1* and *cry8Ea1* genes. Additionally, the *cry2A*, *cry11A*, *cry15A*, and *cry18A* operons have a single promoter, located upstream of the first *orf* operon. No promoter activity was found in the intergenic region between the *orf1* and *cry* genes (*cry2A*, *cry11A*, *cry15A*, and *cry18A*) (8, 11, 25, 30), suggesting that the *cry8Ea1* operon promoter is a novel *cry* gene promoter. To our knowledge, this is the first report of the existence of a promoter in the intergenic region between the *orf1* and *cry* genes.

P_{orf1} is a σ^E-dependent promoter. The time course of P_{orf1} activity increased sharply at T_3, suggesting the possibility of a sporulation-specific sigma factor. Therefore, the σ^E and σ^K dependence of P_{orf1} was tested. The recombinant plasmid pHTPorf1 was introduced into the HDΔsigE and the HDΔsigK strains, respectively. The β-galactosidase assay showed that P_{orf1} activity was

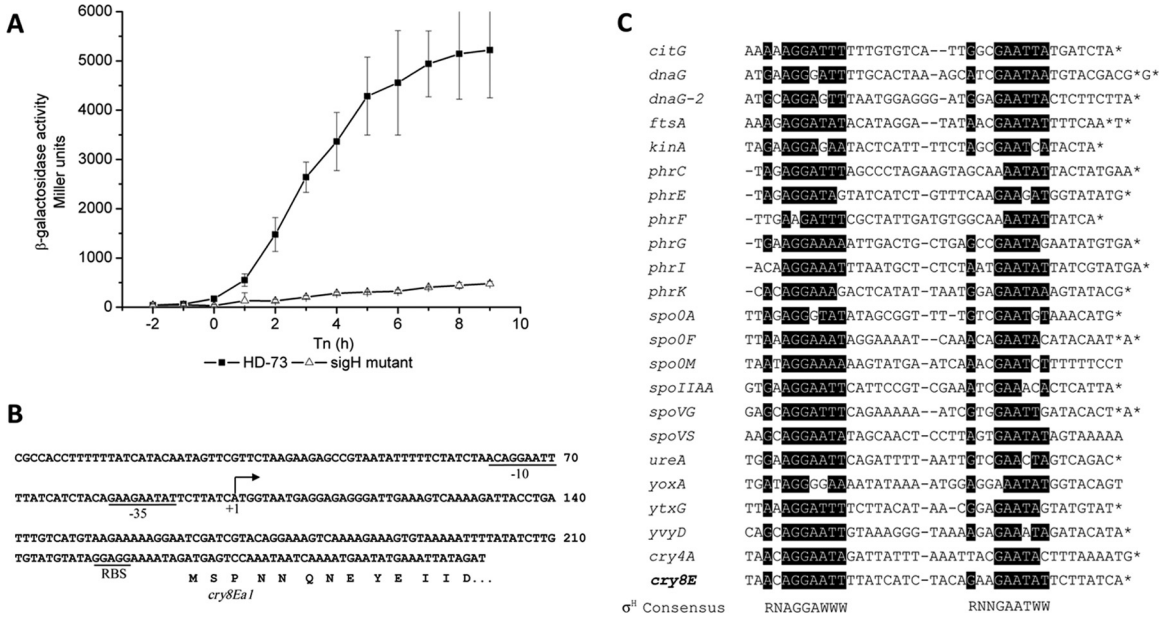

FIG 4 (A) Analysis of P_{cry8E} promoter activities in the HD-73 wild-type strain (■) and the *sigH* mutant (△). The promoter-directed synthesis of β-galactosidase in the strains was determined at the indicated times after growing the cells in SSM at 30°C. Each value represents the mean for at least three independent replicates. (B) The promoter region of the operon. Transcriptional initiation (+1) is marked. The putative promoter region of the operon (−35 and −10) and the putative ribosome binding site of *orf1* (RBS) are underlined and marked. "*cry8Ea*" below the arrow indicates the 5′ end of *orf1* and the deduced amino acid sequence. (C) Sequence similarity between the P_{cry8E} promoter and the consensus sequences was obtained by aligning the σ^H-dependent promoters of *B. thuringiensis*. "*" indicates the transcriptional initiation sites. Consensus sequences of σ^H-dependent promoters are presented above and below the alignment, respectively. R is A or G; W is A or T.

almost abolished in the HDΔsigE strain and was similar in the wild-type and HDΔsigK strains before T_{10} (Fig. 3A).

The transcriptional start site was determined by 5′-RACE–PCR to be a single 5′-end G residue located 64 nucleotides (nt) upstream of the *orf1* translational start codon (Fig. 3B). The nucleotide sequences of the −35 and −10 regions of the *orf1* gene are similar to those of some σ^E-dependent *cry* genes previously described (30) (Fig. 3C). Taken together, these results suggested that high-level transcription of the *cry8Ea1* gene is controlled by the σ^E factor during sporulation, similar to regulation of the *cry2A* and *cry15A* genes (8, 25).

P_{cry8E} is a σ^H-dependent promoter. The intergenic region between *orf1* and *cry8Ea1* encodes a promoter with low-level activity at the onset of sporulation (Fig. 2B). The nucleotide sequences of the −35 and −10 regions of the *cry8Ea1* gene are highly similar to those of several σ^H-dependent genes in *Bacillus subtilis* (6) (Fig. 4C). To test whether the *cry8E* gene promoter is σ^H dependent, the plasmid pHTPcry8E was introduced into HDΔsigH. β-Galactosidase activity in the HDΔsigH strain was dramatically lower than that in the wild-type strain (Fig. 4A). The results of 5′-RACE–PCR mapping performed on total RNA isolated from BT185 cells at T_1 indicated that a transcription start site mapped 133 bases upstream of the *cry8Ea1* gene start codon (Fig. 4B). The nucleotide sequence of the promoter region is similar to that of σ^H-dependent genes (7) (Fig. 4C). These results suggested that the P_{cry8E} promoter with low-level activity located in the intergenic region between *orf1* and *cry8Ea1* is controlled by the σ^H factor during the transition phase. The *cry4A* and *cry11A* genes are also weakly transcribed by the σ^H form of RNA polymerase, and the σ^H-dependent promoter overlaps the σ^E-dependent promoter (19, 28). This is not the case with the *cry8Ea1* operon promoter, which is located upstream of two transcriptional start sites of the *cry8Ea1* gene. This work describes the unique character of the *cry8Ea1* operon promoter, which is composed of P_{orf1} and P_{cry8E}, controlled by the σ^E and σ^H factors, respectively.

ACKNOWLEDGMENTS

This work was supported by grants from the Key Project of Chinese National Programs for Fundamental Research and Development (973 Program) (2009CB118902) and State Key Laboratory Foundation for Biology of Plant Diseases and Insect Pests (SKL2011OP04).

REFERENCES

1. **Agaisse H, Lereclus D.** 1994. Expression in *Bacillus subtilis* of the *Bacillus thuringiensis cryIIIA* toxin gene is not dependent on a sporulation-specific sigma factor and is increased in a *spo0A* mutant. J. Bacteriol. **176**:4734–4741.
2. **Agaisse H, Lereclus D.** 1994. 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.
3. **Agaisse H, Lereclus D.** 1995. How does *Bacillus thuringiensis* produce so much insecticidal crystal protein? J. Bacteriol. **177**:6027–6032.
4. **Arnaud M, Chastanet A, Debarbouille M.** 2004. New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, gram-positive bacteria. Appl. Environ. Microbiol. **70**:6887–6891.
5. **Baum JA, Malvar T.** 1995. Regulation of insecticidal crystal protein production in *Bacillus thuringiensis*. Mol. Microbiol. **18**:1–12.
6. **Bravo A, Agaisse H, Salamitou S, Lereclus D.** 1996. Analysis of *cryIAa* expression in *sigE* and *sigK* mutants of *Bacillus thuringiensis*. Mol. Gen. Genet. **250**:734–741.
7. **Britton RA, et al.** 2002. Genome-wide analysis of the stationary-phase sigma factor (sigma-H) regulon of *Bacillus subtilis*. J. Bacteriol. **184**:4881–4890.
8. **Brown KL.** 1993. Transcriptional regulation of the *Bacillus thuringiensis* subsp. *thompsoni* crystal protein gene operon. J. Bacteriol. **175**:7951–7957.
9. **Brown KL, Whiteley HR.** 1988. Isolation of a *Bacillus thuringiensis* RNA polymerase capable of transcribing crystal protein genes. Proc. Natl. Acad. Sci. U. S. A. **85**:4166–4170.

10. **Brown KL, Whiteley HR.** 1990. Isolation of the second *Bacillus thuringiensis* RNA polymerase that transcribes from a crystal protein gene promoter. J. Bacteriol. **172**:6682–6688.
11. **Dervyn E, Poncet S, Klier A, Rapoport G.** 1995. Transcriptional regulation of the *cryIVD* gene operon from *Bacillus thuringiensis* subsp. *israelensis*. J. Bacteriol. **177**:2283–2291.
12. **Du C, Nickerson KW.** 1996. *Bacillus thuringiensis* HD-73 spores have surface-localized Cry1Ac toxin: physiological and pathogenic consequences. Appl. Environ. Microbiol. **62**:3722–3726.
13. **Du L, et al.** 2011. Characterization of *Bacillus thuringiensis sigK* disruption mutant and its influence on activation of *cry3A* promoter. Acta Microbiol. Sin. **51**:1177–1184. (In Chinese.)
14. **Guo S, et al.** 2009. Crystal structure of *Bacillus thuringiensis* Cry8Ea1: an insecticidal toxin toxic to underground pests, the larvae of Holotrichia parallela. J. Struct. Biol. **168**:259–266.
15. **Lambert B, et al.** 1996. A *Bacillus thuringiensis* insecticidal crystal protein with a high activity against members of the family Noctuidae. Appl. Environ. Microbiol. **62**:80–86.
16. **Lereclus D, Arantes O, Chaufaux J, Lecadet M.** 1989. Transformation and expression of a cloned delta-endotoxin gene in *Bacillus thuringiensis*. FEMS Microbiol. Lett. **51**:211–217.
17. **Miller JH.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
18. **Perez-Garcia G, Basurto-Rios R, Ibarra JE.** 2010. Potential effect of a putative sigma(H)-driven promoter on the over expression of the Cry1Ac toxin of *Bacillus thuringiensis*. J. Invertebr. Pathol. **104**:140–146.
19. **Poncet S, Dervyn E, Klier A, Rapoport G.** 1997. Spo0A represses transcription of the *cry* toxin genes in *Bacillus thuringiensis*. Microbiology **143**(Pt 8):2743–2751.
20. **Schaeffer P, Millet J, Aubert JP.** 1965. Catabolic repression of bacterial sporulation. Proc. Natl. Acad. Sci. U. S. A. **54**:704–711.
21. **Shu C, et al.** 2009. Characterization of two novel *cry8* genes from *Bacillus thuringiensis* strain BT185. Curr. Microbiol. **58**:389–392.
22. **Wang G, et al.** 2006. Engineered *Bacillus thuringiensis* GO33A with broad insecticidal activity against lepidopteran and coleopteran pests. Appl. Microbiol. Biotechnol. **72**:924–930.
23. **Wasano N, et al.** 2005. Cloning and characterization of a novel gene *cry9Ec1* encoding lepidopteran-specific parasporal inclusion protein from a *Bacillus thuringiensis* serovar *galleriae* strain. Can. J. Microbiol. **51**:988–995.
24. **Wei J, et al.** 2008. Construction and characteristics of the *sigE* mutation in *Bacillus thuringiensis*HD-73. Microbiology **35**:1581–1586. (In Chinese.)
25. **Widner WR, Whiteley HR.** 1989. Two highly related insecticidal crystal proteins of *Bacillus thuringiensis* subsp. *kurstaki* possess different host range specificities. J. Bacteriol. **171**:965–974.
26. **Wong HC, Schnepf HE, Whiteley HR.** 1983. Transcriptional and translational start sites for the *Bacillus thuringiensis* crystal protein gene. J. Biol. Chem. **258**:1960–1967.
27. **Wu D, Cao XL, Bai YY, Aronson AI.** 1991. Sequence of an operon containing a novel delta-endotoxin gene from *Bacillus thuringiensis*. FEMS Microbiol. Lett. **65**:31–35.
28. **Yoshisue H, Ihara K, Nishimoto T, Sakai H, Komano T.** 1995. Expression of the genes for insecticidal crystal proteins in *Bacillus thuringiensis*: *cryIVA*, not *cryIVB*, is transcribed by RNA polymerase containing sigma H and that containing sigma E. FEMS Microbiol. Lett. **127**:65–72.
29. **Yu H, Zhang J, Huang D, Gao J, Song F.** 2006. Characterization of *Bacillus thuringiensis* strain Bt185 toxic to the Asian cockchafer: *Holotrichia parallela*. Curr. Microbiol. **53**:13–17.
30. **Zhang J, Schairer HU, Schnetter W, Lereclus D, Agaisse H.** 1998. *Bacillus popilliae cry18Aa* operon is transcribed by sigma E and sigma K forms of RNA polymerase from a single initiation site. Nucleic Acids Res. **26**:1288–1293.