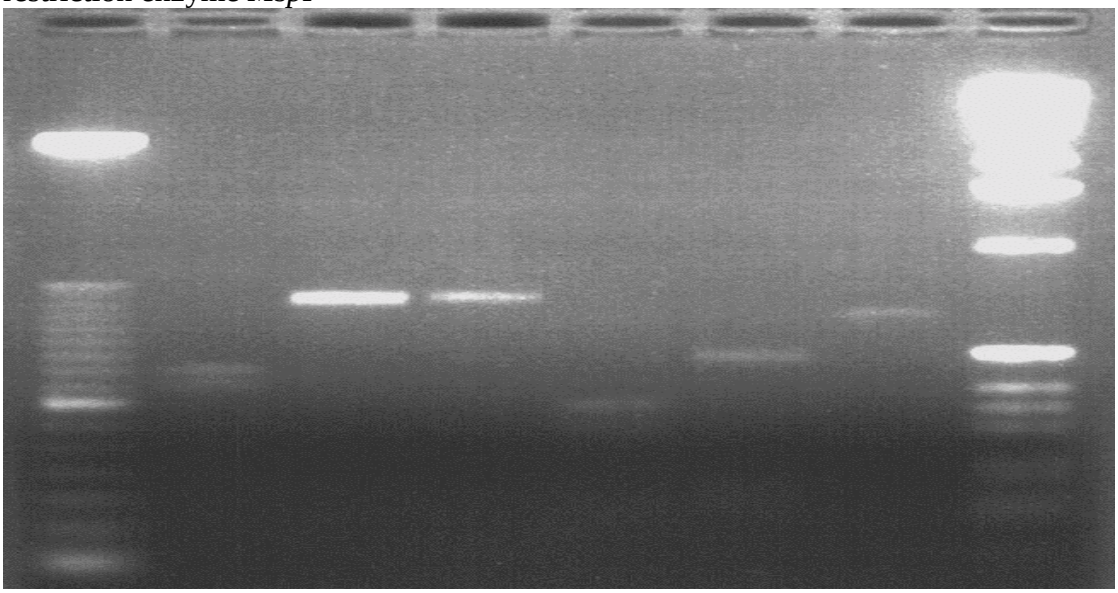
The restriction enzyme *MspI* was used to perform a restriction digestion on three of the CryBP2 PCR reactions in order to compare restriction patterns and to further demonstrate differences in the amplicons. *MspI* was chosen based on the restriction map of the published *cry18Aa1* gene from strain H1 and location of the CryBP2 primers. There are two *MspI* restriction sites in the region between the CryBP2 primers and restriction fragments of approximately 160, 475, and 140 bp were expected. The Central and South American *P. popilliae* strains 381 and 492 and *P. lentimorbus* strain 266 were chosen to represent the three amplicon sizes. *P. popilliae* strain H1, the *P. popilliae* type strain, ATCC 14706, and the *P. lentimorbus* type strain, ATCC 14707, were included for comparison to the Central and South American amplicons. The restriction digestion of the 806 bp amplicon of strain H1 yielded one band of approximately 450 bp (Fig. 4, lane 2). The 660 bp amplicons for *P. popilliae* strains ATCC 14706 and 381 each had a single band of approximately 775 bp (Fig. 4, lanes 3 and 4, respectively). The 1100 bp amplicon for *P. popilliae* strain 492 gave one band of approximately 350 bp (Fig. 4, lane 5) and *P. lentimorbus* strains 266 and ATCC 14707 each gave a single band of approximately 500 bp and 700 bp, respectively (Fig. 4, lanes 6 and 7, respectively). Although some fragments may not have resolved well or small restriction fragments may have migrated off the gel, there are noticeable differences in the sizes of the digests indicating possible variability in the parasporal genes.

Figure 4. Restriction digestion of *P. popilliae* and *P. lentimorbus* CryBP2 amplicons using the restriction enzyme *MspI*



Lane 1, 50 bp DNA ladder; Lane 2, H1; Lane 3, ATCC 14706; Lane 4, 381; Lane 5, 492; Lane 6, 266; Lane 7, ATCC 14707; Lane 8, 1 kb DNA ladder

CryBP2 PCR product sequencing. *Paenibacillus popilliae* strains 381 and 492 and *P. lentimorbus* strain 266 were chosen to represent the three amplicon sizes from the CryBP2 PCR reactions. The PCR products from these strains were sequenced (1) and compared to the published *cry18Aa1* gene sequence (4). The sequence obtained for the amplicons for strains 381 and 266 differed by 67 bp (6 insertions, 15 deletions and 46 mutations) and 47 bp (6 insertions, 3 deletions and 38 mutations), respectively, from the published sequence (Fig. 5). The amplicon for strain 492 contains a 453 bp insert that is not found in the published sequence. The insert appears to start in the intergenic region between the *orf1* and *cry* genes. A BLAST search on the National Center for Biotechnology Information web site found that part of the insert (underlined in Fig. 5) aligns with a region at the 5' end of the *orf1* gene (bp 132 through 241) of the published sequence. The sequence for *P. lentimorbus* ATCC 14707 shows no similarity to the published sequence.

Figure 5. Sequence alignment of *Paenibacillus popilliae* and *P. lentimorbus cry* genes

```
                472
cry18Aa1        ATTTCTATTTGCTGCACAGAAAGTATCTGTATAGATCATG
381             ---G-A---G-T---G--------------G--------A

                512
cry18Aa1        TACTGAAATGCAGTGTGGAAACCAGCCCCCATCATCATGT
381             --------------------CA—-T-------------T-
266                ------------------CA—-T-------------T-
492                -------T-----A—-TCA-GA------------CT-

                552
cry18Aa1        GGACTGCCATCATGTGGTAGTTCATGATTTGAAAGCAATC
381             ----------------------------------------
266             ------------------G---------------------
492             ------------------------------------GA-

                592
cry18Aa1        CCAATCCGTGAAGATCATTGCCGGTTCGTTAAAGTTACGG
381             ---------------T---------A--------------
266             ----------------------------------------
492             --CG-----A----------T-A--------------T--

                632
cry18Aa1        GGAACTTTCAATTTCATTATGTAAAGGATTTG1TAAACCGA
381             ---------------------------------1----T---
266             -----------G---------------------1--------
492             ---------------G-------------C--1--------

                672
cry18Aa1        AACACAGGCTGGAATGA CCGAGGCGATTTGGATAGATTT
381             ----------------- ----------------------
266             ----------------- ----------------------
492             --A-----T-------- ::::::::::::::::::::::
                                  3

                712                    RBS
cry18Aa1        GAATGCTCATCATATGAAGGAGGCTATTGGTATG2AACAAT
381             A-C-------A-----------------------2------
266             ---------------------------------2------
492             :::::::::::::::::::::::::::::::: ::::::
```

```
                752
cry18Aa1        AATTTTAATGGTGGAAATAATACAGGAAATAACTTTACTG
381             --C-G------:::------:::::::::-:::-------
266             --C-G------:::--------------------------
492             ::::::::::::::::::::::::::::::::::::::::

                792
cry18Aa1        GAAATACTCTAAGCAACGGAATTTGTACGAAAAAAAATAT
381             ----------------------------------------
266             -------C—-G----------------------------A
492             ::::::::::::----------------------------A

                832
cry18Aa1        GAAAGGAACCCTAAGCAGAACTGCTATATTTTCAGATGGG
381             -------------------R--------G---------C-
266             ----------------------------G---------C-
492             ----------------------------G---------C-

                872
cry18Aa1        ATTAGTGATGATTTAATTTGTTGTCTAGATCCTATATATA
381             ----------------------------A------------
266             ----------------------------A------------
492             ----------------------------A------------

                912
cry18Aa1        ACAATAACGATAACAATAA::::::CGATGCTATTTGTGA
381             ---G—-M------------CAATAW---------------
266             ---G---------------CAATAA---------------
492             ---G---------------CAATAA---------------

                946
cry18Aa1        TGAGTTAGGTTTAACTCCAATAGATAACAATACGATATGC
381             ----------------------------------------
266             ----------------------------------------
492             ----------------------------------------

                986
cry18Aa1        AGTACTGATTTTACTCCCATAAATGTAATGAGAACAGATC
381             ----------A----------------------------
266             ----------A----------------------------
492             ----------A------------C---------------

                1026
cry18Aa1        CTTTTCGCAAGAAATCAACACAAGAACTCACAAGGGAATG
381             ----------------------------T-T---------
266             --------G------------------T-T---------
492             ----------------------------T-T---------
```

```
                1066
cry18Aa1        GACAGAATGGAAAGAAAATAGTCCTTCTTTGTTTACACCG
381             ----------------------------------------
266             ----------------------------------------
492             ----------------------------------------

                1106
cry18Aa1        GCAATTGTAGGTGTCGTTACCAGTTTTCTTCTTCAATCAT
381             ------------AC---------------C------CA-A
266             ------------A------------A---C---A—-CA-A
492             ------------AC--------------------WCA-A

                1146
cry18Aa1        TAAAAAAACAAGCAACTAGCTTTCTTTTAAAAACTTTGAC
381             --GC----GT—A---T-G--GC---A--G---C
266             —-GC----GT-A---T-G--GC---A—-G---C
492             —-GC----GT-A---T-G--GC---A--G---C-------

                1186
cry18Aa1        AGACCTATTATTTCCTAATAACAGTTCGTTAACGATGGAA
492             —A----T---------GG-GG----A-------A------

                1226
cry18Aa1        GAGATTTTACGAGCCACGGAAC
492             ----------------------
```

- = identical base

: = missing base

[1]End of *orf1*

[2]Start codon of *cry* gene

[3]453 bp insert found in strain 492 sequence but not found in other sequences

GATGTCGGTGTAAGTTGTCCTGCGGGTGACGAAAGTTGGCCCCTTAATACGATGAAGAT
TCAGGGAGGCTCATGAGAGCTCTCCCTGAATCTGCTTCACCGGATCTTCCGGCTCAAAG
TATCTGGATAGGACGGATACTTTGAAGTGGCGGAAACAATCGAAAGACTGCATCCAACG
TTGTGCGTGTTGTGCGTTGGACTTGATCCTTGGGGATGGGAACTTTATTGCCTTAATCT
GCATGGAATGGTTCAAAACTGAATAGAATGGATTAAGCAGAATGCATCTTATTCACCAA
ATACAGTTAGAAAATAGGGAAAGGAGGCGATGA**AAAGG**AAATGAATACAAATCATGATC
AACTGGTGAGTTCACTTCATTCGAATTCAGAATTACCACCCCATTGTATACCGGAAGGA
TATCAAATAGTGCCGTTTTGTTGTGTGATTCCGATCCCGC

Discussion

In 1997, Zhang *et al*. (4) cloned and sequenced the parasporal gene, *cry18Aa1*, from the European *P. popilliae* strain H1. Although the role of the parasporal protein in pathogenesis is debatable, the *cry18Aa1* gene is of interest because of structural and sequence similarities to *Bacillus thuringiensis cry* genes, which are toxic to insects of the Lepidoptera, Diptera, and Coleoptera families (3). Amplification of a portion of the *cry* genes using the CryBP2 primers in the Mexican and Central and South American strains of *P. popilliae* and *P. lentimorbus* indicated possible variability in parasporal proteins among the strains. This finding was interesting because Rippere-Lampe (2) reported that all North American *P. popilliae* strains screened for the *cry* gene with the CryBP2 primers produced amplicons of the approximately the same size, suggesting no variability in the parasporal proteins. Differences in the restriction patterns of the CryBP2 digests for the *P. popilliae* H1 strain, the *P. popilliae* type strain, the *P. lentimorbus* type strain, and three of the Central and South American strains (*P. popilliae* strains 381 and 492 and *P. lentimorbus* strain 266), which represented the variable amplicon sizes, further supported the idea that there was variation in the region of the parasporal gene amplified (Fig. 4).

In order to support my findings with the CryBP2 primers, the South American *P. popilliae* strains 381 and 492 and the South American *P. lentimorbus* strain 266 amplicons were sequenced and compared to the published *cry18Aa1* gene. Although I did not have enough sequencing data to account exactly for the size differences in strains 381 and 266, I could determine that the sequences are similar to the published sequence and that strain 266 differs from strain 381 by 30 bp (Fig. 5). Strain 266 differs from the published sequence by 47 bp; strain 381 differs from the published sequence by 67 bp (Fig. 5). Interestingly, the insertions and deletions in the 266 and 381 sequences occur in multiples of three. Even though these insertions

or deletions may change the amino acids, the reading frames are conserved. The mutations, insertions, and deletions in the sequences do not affect paraspore formation and show that there is variability in the parasporal proteins. A 453 bp insert explains the size difference in the amplicon for strain 492. Based on the alignment of the sequence with the published sequence, the insert appears to begin in the intergenic region between the *orf1* and *cry* genes (Figs. 1 and 5) and the sequence does not align again until within the *cry* gene. A BLAST search revealed that approximately 114 bp at the 3'end of the insert (underlined in Fig. 5) are a duplication of bp 132 through 214 at the 5' end of the *orf1* gene. The ribosome binding site (RBS) for the *orf1* gene begins at bp 131. Both of the amplicon sequences for strains 266 and 381 have a RBS for the *cry* gene that is identical to the published gene (Fig. 5), however the amplicon for strain 492 lacks a RBS sequence similar to the *cry18Aa1* gene RBS. It seems reasonable to suggest that the duplicated sequence would provide a RBS for the *cry* gene in strain 492. However, more studies are necessary to show that this is true. The suggested RBS is highlighted in Fig. 5. The insertion does not affect parasporal formation because the paraspore body was observed in a smear of infected insect hemolymph.

The CryBP2 and CryBP4 primers detected the *cry* gene in all of the *P. lentimorbus* strains. Rippere-Lampe (2) reported that the parasporal gene was not detected in the paraspore-forming strains of *P. lentimorbus* from North America when screened with the CryBP2 primer pair. The inability to detect the *cry* gene in the North American strains of *P. lentimorbus* suggests variability in the regions where the primers anneal. I was also able to detect the cry gene in the *P. lentimorbus* type strain ATCC 14707 with both the CryBP2 and CryBP4 primers (Figs 2 and 3). Detection of the paraspore gene in the *P. lentimorbus* type strain was unexpected because it has been characterized by its inability to produce a parasporal body. The CryBP2 amplicon

sequence for the *P. lentimorbus* type strain ATCC 14707 does not show any similarity to the published *cry18Aa1* sequence. This may explain the lack of parasporal body formation during sporulation; perhaps the parasporal protein is not synthesized, is not stable, or does not aggregate. A BLAST search did not reveal any significant similarity to any other published sequences. Since the CryBP2 and CryBP4 primers were able to detect the parasporal gene, I would suggest that the entire parasporal gene be sequenced and compared to the published sequence in order to determine if any similarity exists between the published *cry18Aa1* gene and the ATCC 14707 *cry* gene. Sequencing of the complete gene may also explain why this strain does not produce a paraspore during sporulation.

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