CHAPTER FOUR

Characterization of parasporal genes in *Paenibacillus popilliae* and *Paenibacillus lentimorbus*

Abstract

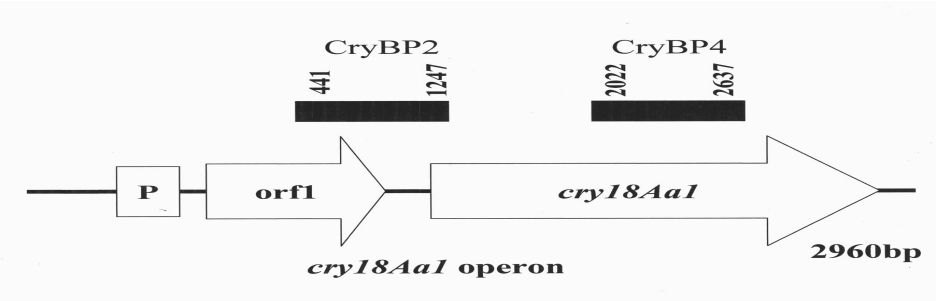
The parasporal gene, *cry18Aa1*, was cloned and sequenced by Zhang *et al*. (4) from the European *Paenibacillus popilliae* strain H1 isolated from *Melolontha melolontha*. The *P. popilliae* and *P. lentimorbus* strains from Mexico and Central and South America were screened for the presence of a gene resembling that of the published *cry18Aa1* gene sequence. PCR was used to amplify two different regions of the *cry* operon. One primer pair, CryBP2, detected the parasporal gene in 17 of the 21 *P. popilliae* strains and in all ten of the *P. lentimorbus* strains. The CryBP2 PCR primers produced amplicons of three sizes; the *P. popilliae* strains yielded amplicons of two different sizes (approximately 660 and 1100 bp) and the *P. lentimorbus* strains yielded an amplicon of a single size (approximately 750 bp). A second primer pair, CryBP4, detected the *cry* gene in 20 of the 21 *P. popilliae* strains and in all ten of the *P. lentimorbus* strains. Both the *P. popilliae* and *P. lentimorbus* strains showed the same size amplicons for the CryBP4 primers (616 bp). Three CryBP2 PCR products, representing the three different size amplicons, were sequenced and compared to the published *cry18Aa1* gene sequence. Sequence alignments showed that the parasporal gene regions amplified with the CryBP2 primers in two of the Central and South American strains are similar to the published sequence. However, one of the CryBP2 PCR amplicons that was sequenced has a 453 bp insert that is not found in the published sequence of the parasporal gene of strain H1. Further analysis revealed that the insert

contains a region at the 3’ end that shows significant similarity to the 5’ end of the *orf1* gene in the *cry* operon.

Results

Detection of the *cry* operon. Two sets of primers were used to detect the *cry* operon in the Mexican and Central and South American strains of *P. popilliae* and *P. lentimorbus*. The structure of the *cry18Aa1* operon is shown in Figure 1. One primer pair, CryBP2, amplifies a region from bp 441 to bp 1247 and begins in the 3’ end of the *orf1* gene and ends in the 5’ end of the *cry18Aa1* gene (2). The second primer pair, CryBP4, amplifies a region from bp 2022 to bp 2637 and is located towards the 3’ end of the *cry18Aa1* gene.

Figure 1. *cry18Aa1* operon and location of primers used to detect *cry* operon in *P. popilliae* and *P. lentimorbus* strains



The results of the PCR reactions are shown in Table 1. Based on the published sequence, strain H1 was expected to produce an 806 bp fragment with the CryBP2 primers and a fragment size of 616 bp with the CryBP4 primers. The amplicon from the CryBP2 primers for the *P. popilliae* type strain ATCC 14706 was sequenced and compared to the published sequence by Rippere-Lampe (2) and was used in this study as a control for comparing amplicon sizes. ATCC 14706

produces a 660 bp fragment (Fig. 2, lane 3). When the Mexican and Central and South American strains were tested with the CryBP2 primers, 11 of the *P. popilliae* strains produced PCR fragments of approximately 660 bp (e.g. Fig. 2, lane 4) and six of the *P. popilliae* strains produced PCR fragments of about 1100 bp (e.g. Fig. 2, lane 5). Four of the *P. popilliae* strains did not produce PCR fragments under the conditions tested. All of the *P. lentimorbus* strains from Central and South America produced amplicons of approximately 750 bp (e.g. Fig. 2, lane 6). The *P. lentimorbus* type strain, ATCC 14707, was also tested with the CryBP2 primers and an amplicon of about 600 bp was seen (Fig. 2, lane 7). When the CryBP4 primers were used to amplify the parasporal gene in the Mexican and Central and South American strains, all *P. popilliae* and *P. lentimorbus* strains, except 470, produced amplicons of approximately 616 bp (Fig. 3); strain 470 did not produce a PCR product under the conditions tested. The type strain for *P. lentimorbus* produced an amplicon of approximately 575 bp with the CryBP4 primers (Fig. 3, lane 11).

Table 1. Detection of *cry* operon in *Paenibacillus popilliae* and *P. lentimorbus* using CryBP2 and CryBP4 primers and approximate sizes of amplicons produced

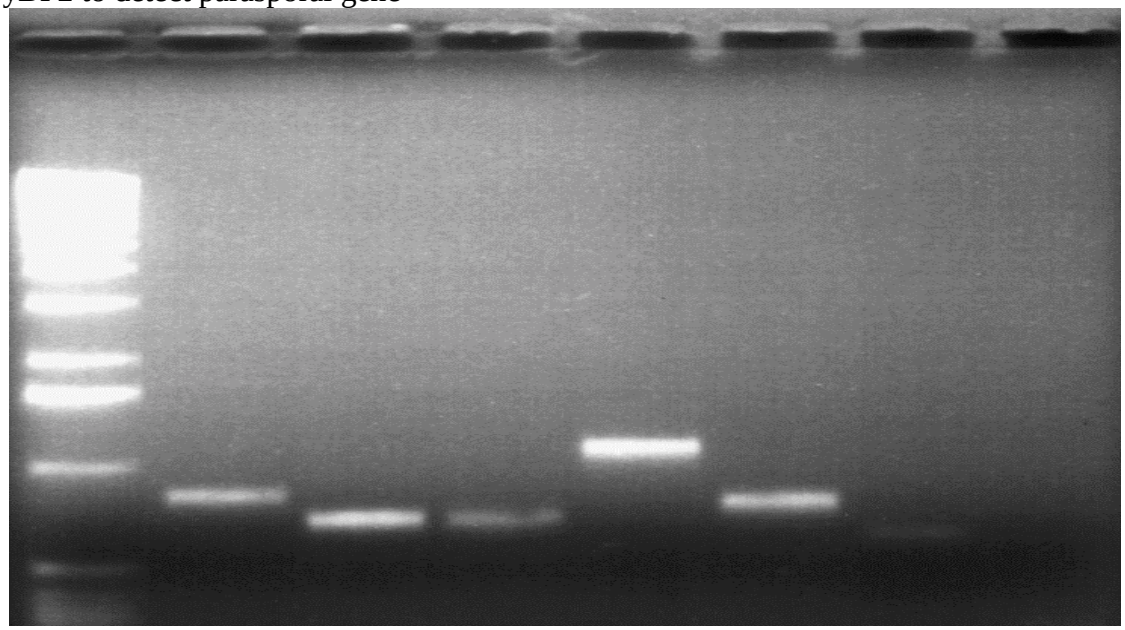
Strain	CryBP2	CryBP4
P. popilliae		
ATCC 14706	+[1]	+[5]
H1	+[2]	+[5]
381	+[1]	+[5]
382	+[1]	+[5]
394	+[1]	+[5]
395	+[1]	+[5]
470	nd	nd
479	nd	+[5]
491	+[3]	+[5]

492	+[3]	+[5]
493	+[3]	+[5]
494	+[3]	+[5]
499	+[3]	+[5]
502	+[3]	+[5]
503	+[1]	+[5]
504	+[1]	+[5]
508	+[1]	+[5]
510	nd	+[5]
518	+[1]	+[5]
522	+[1]	+[5]
524	+[1]	+[5]
525	nd	+[5]
527	+[1]	+[5]
P. lentimorbus		
ATCC 14707	+[6]	+[7]
266	+[4]	+[5]
283	+[4]	+[5]
285	+[4]	+[5]
289	+[4]	+[5]
290	+[4]	+[5]
292	+[4]	+[5]
299	+[4]	+[5]
393	+[4]	+[5]
495	+[4]	+[5]
497	+[4]	+[5]

[nd] not detected

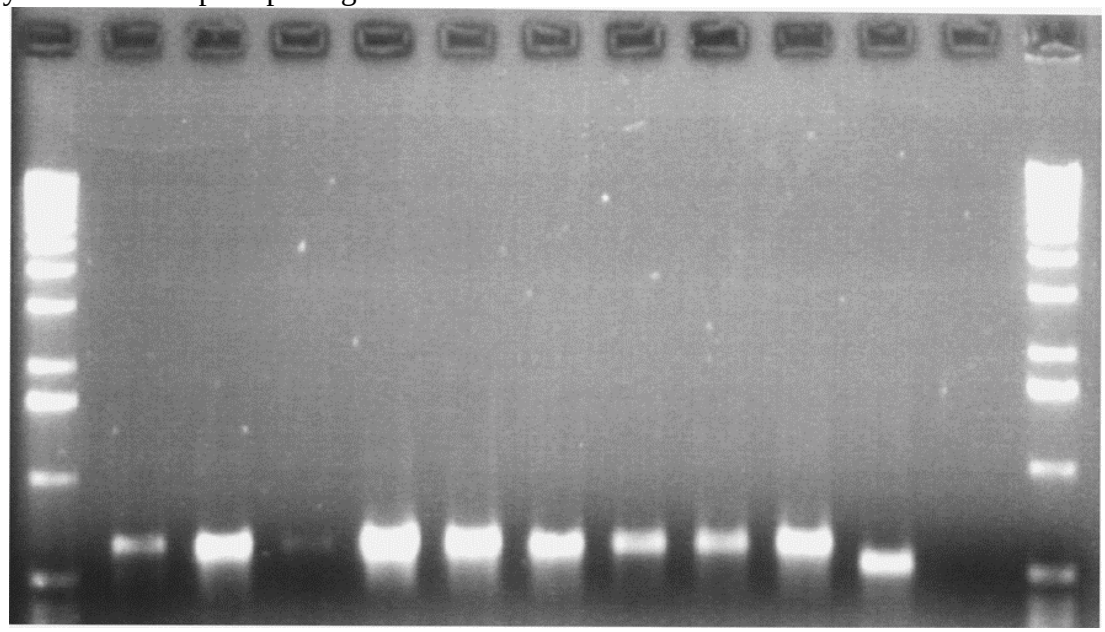
[1]CryBP2 amplicon size approximately 660 bp; [2]CryBP2 amplicon size 806 bp; [3]CryBP2 amplicon size approximately 1100 bp; [4]CryBP2 amplicon size approximately 750 bp; [5]CryBP4 amplicon size approximately 616 bp; [6]CryBP2 amplicon size of 600 bp; [7]CryBP4 amplicon size of 575 bp

Figure 2. PCR products amplified from the DNA of *P. popilliae* and *P. lentimorbus* using primer pair CryBP2 to detect parasporal gene



Lane 1, 1 kb DNA ladder; Lane 2, H1; Lane 3, ATCC 14706; Lane 4, 381; Lane 5, 492; Lane 6, 266; Lane 7, ATCC 14707; Lane 8, negative control (no DNA)

Figure 3. PCR products amplified from the DNA of *P. popilliae* and *P. lentimorbus* using primer pair CryBP4 to detect parasporal gene



Lane 1, 1 kb DNA ladder; Lane 2, ATCC 14706; Lane 3, 381; Lane 4, 492; Lane 5, 518; Lane 6, 525; Lane 7, 266; Lane 8, 289; Lane 9, 393; Lane 10, 497; Lane 11, ATCC 14707; Lane 12, negative control (no DNA); Lane 13, 1 kb DNA ladder