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Using DNA Microarrays for Assessing Crystal Protein Genes in *Bacillus thuringiensis*

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A DNA microarray (cryArray) was designed to identify cry gene contents of *Bacillus thuringiensis* (Bt) strains. A consentaneous approach was used in which multiple DNA *cry*-specific probes must all produce a positive hybridization signal to confirm a cry gene's presence. The immobilized cry-specific oligonucleotide probes agreed with the cry contents of known or PCR-validated Bt strains. In one strain, the cryArray was able to detect the presence of a novel cry1I gene. Since the cryArray can replace hundreds of individual PCR reactions, it should become a valuable tool for fast screening of new Bt isolates presenting interesting insecticidal activities.

Although many Cry proteins are structurally and functionally similar, the diversity of Cry toxins and their insecticidal spectra is immense. More than 280 different Cry toxins are organized into 46 primary ranks based on amino acid similarities (1). Cry1 toxins are the largest and best known family having over 130 entries in the Cry databank. Since many Bt strains typically harbor one to nine *cry* genes, some of which are known to be cryptic (2), it is clear that to assess the complete *cry* gene content of unknown Bt strains, a technology possessing good parallel processing capabilities is required. Since DNA microarrays possess this ability to simultaneously hybridize with thousands of different genes, a *cry* gene microarray (*cryArray*) was designed which contained *cry1* gene-specific oligonucleotides, each spotted and immobilized in triplicate. Various other *cry* gene primary ranked classes were also included (Fig. 1).

All oligonucleotides were spotted in triplicate (horizontally). The dotted box on the left represents primary ranked *cry* genes. The dashed box on the right represents the different secondary ranked *cry1* gene families. Hybridizations performed with 750 nags (5 ng/mm^2) of Cy5-labeled amplified genomic DNA gave sufficiently clear results by producing strong fluorescent signals. The array was constructed using a consentaneous approach so that the presence of any given gene is confirmed only if all the secondary rank probes and all higher rank probes targeting different regions within the gene produced positive hybridization signals. Initial hybridizations were carried out using well-characterized laboratory or commercial Bt single gene strains (HD-73 or Bt subsp. *kenyae*; *cry1Ac* or *cry1E*). Known multi-gene strains (HD-1 or HD-133) were subsequently used to validate this approach (data not shown).

By creating a series of family primers, the *cryArray* also possessed the ability to detect unknown gene variants within a particular family. An illustration of this is provided in Fig. 2. In this case, the *cry1I* family primers (secondary ranked) produced a positive hybridization signal from genomic DNA of an unknown Bt strain (IB360) obtained from a Mexican culture collection (3), yet all of the tertiary oligonucleotide probes were negative. The family primers were used to amplify the gene which was sequenced and confirmed to be a novel *cry1I* gene. As shown in Fig. 3, sufficient homology existed to the family primers to result in positive hybridization signals, whereas, apart from one of two *cry1Ia* probes, insufficient similarity at the tertiary level resulted in negative signals.

By adding oligonucleotide probes to those present on the existing *cryArray*, probing the array with fluorescently labeled DNA-free RNA allowed the determination of *cry* gene expression (Fig. 4). Figure 4 shows all three *cry1A* genes producing positive signals with Cy-5 labeled mRNA, whereas the DNA *cry* gene detection probes remained negative.

In conclusion, we found that DNA microarrays can generally provide single oligo *cry* gene discrimination

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down to the secondary rank but that a consentaneous or 'multiple oligo' approach is needed to achieve discrimination down to the tertiary rank level. Finally, by incorporating complementary oligonucleotide probes, *cry* gene activity (expression) could be monitored by direct RNA labeling which allows discrimination between active versus cryptic or disrupted genes.

References

1. **Crickmore, N.** 2006 Full list of delta-endotoxin. URL: http://www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.html. Accessed Feb. 2006.
2. **Masson, L., M. Erlandson, M. Puzstai-Carey, R. Brousseau, V. Juárez-Pérez, and R. Frutos.** 1998. A holistic approach for determining the entomopathogenic potential of *Bacillus thuringiensis* strains. Appl. Environ. Microbiol. **64:**4782-4788.
3. **Bravo, A., S. Sarabia, L. Lopez, H. Ontiveros, C. Abarca, A. Ortiz, M. Ortiz, L. Lina, F. J. Villalobos, G. Peña, M.-E. Nuñez-Valdez, M. Soberón, and R. Quintero.** 1998. Characterization of *cry* genes in a Mexican *Bacillus thuringiensis* strain collection. Appl. Env. Microbiol. **64:**4965-4972.

FIG. 1. *cryArray* key of the tertiary *cry1* genes.

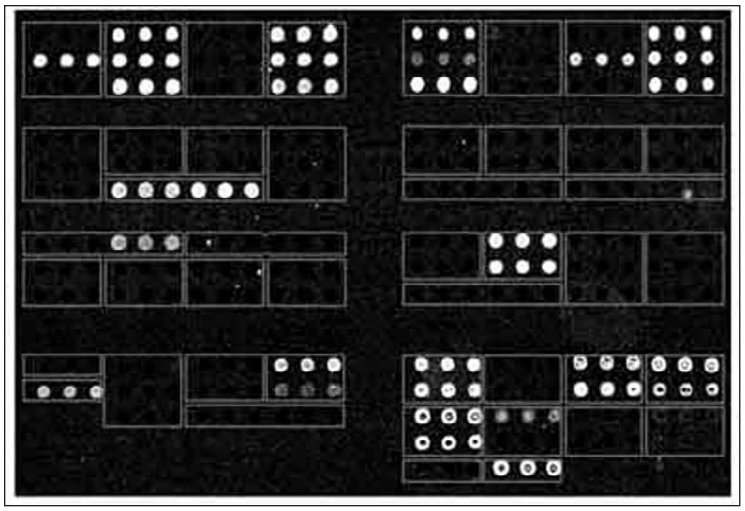

FIG. 2. Scanned image of microarrays hybridized with Cy5-labeled genomic DNAs from *B. thuringiensis* strain IB360. Probes belonging to the same *cry* gene target are grouped inside the individual squares.

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IB360           tattgcgggtaaaatacttgg C accctaggcgttccttttgcaggacaag
cry1Ia          tattgcgggtaaaatacttgg T accctaggcgttccttttgcaggacaag

IB360           tatt C ccaagctatgatacac TTG t A tatccaattaaaactac TT c T ca
cry1Ia          tatt T ccaagctatgatacac AAA t G tatccaattaaaactac AG c C ca

IB360           g G gttgatttT cattggaaatt CGT cac A c AT cc G at C gcatctgataat
cry1Ib          g A gttgatttC cattggaaatt TCC cac G c TA cc A at A gcatctgataat

IB360           aacac AA g G gc T aggagtgt T gtcaaga G ccaatatatcgcattagaa T
cry1Ib/c        aacac TC g A gc G aggagtgt A gtcaaga A ccaatatatcgcattagaa C

IB360           cgtacaaatacaattgagccaaatagcattacacaaataccattagtaaa
cry1I general   cgtacaaatacaattgagccaaatagcattacacaaataccattagtaaa

IB360           agat A taccaattccatacgtcaattaacggtaaagctattaatcaaggta
cry1I general   agat T taccaattccatacgtcaattaacggtaaagctattaatcaaggta
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FIG. 3. Sequence comparison of the *cry1I* amplicon from Bt strain IB360 to *cry1I*-specific immobilized *cryArray* probes. Differences between the *cry1I* gene amplified from strain IB360 and the immobilized specific secondary or tertiary *cry1I*-derived gene sequence are shown in large, boldfaced capital letters.

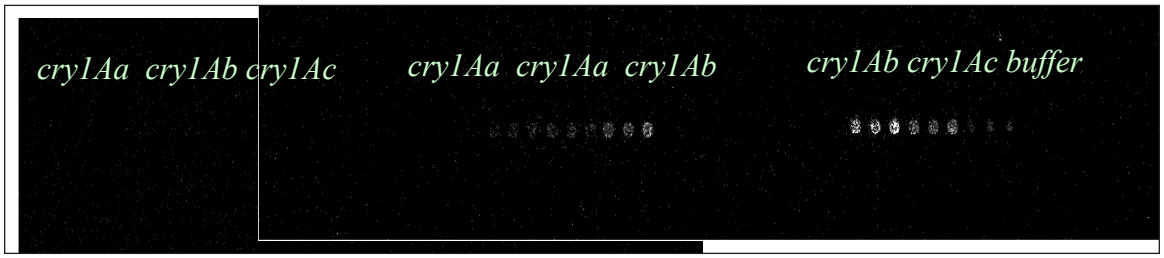

FIG. 4. Partial image of a *cryArray* hybridized with total RNA from Bt strain HD-1. The white box on the left contains negative strand-specific oligonucleotides normally used to detect *cry1Aa*, *b* and *c* genes. The probes on the right represent complementary probes to the *cry1A* genes on the left.