

A SIMPLE, RAPID, AND SENSITIVE ASSAY FOR EVALUATING *BACILLUS THURINGIENSIS* STRAINS FOR THEIR INSECTICIDAL ACTIVITY TOWARD TARGET INSECTS

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ABSTRACT

Bacillus thuringiensis (Bt) is an aerobic, gram-positive bacterium that is used as a biopesticide for the control of lepidopteran, dipteran, and coleopteran insect pests. The insecticidal activity of Bt is mainly due to crystal (Cry) proteins produced by the bacterium during sporulation. Bt has a worldwide distribution, and a large number of different Bt strains have been isolated. These individual strains produce a variety of insecticidal Cry proteins, each of which is specific to a small group of insect species. Insect bioassays are currently used to detect and measure the insecticidal activity of Bt strains and purified insecticidal proteins derived from new Bt isolates. These bioassays are time consuming and require relatively large amounts of materials. Many factors can affect their results, including temperature, type of diet and feeding periods of the insects, and methods for evaluating the insecticidal activity. Furthermore, many insects are not very sensitive to purified Cry proteins obtained from Bt without the synergistic effect of spores, so these assays rely on assessment of growth inhibition rather than toxicity. Alternative techniques such as voltage clamping and use of cell lines or transfected cells require sophisticated equipment and expensive reagents. Receptor-based assays may not be reliable, because it has been established that binding is not sufficient for insecticidal toxicity.

During the course of the study of the mode of action of Bt in the gypsy moth, *Lymantria dispar*, it was unexpectedly discovered that the insecticidal Cry proteins induced rapid release of a membrane-bound aminopeptidase-N (APN) into the gut fluid. The amount of soluble APN can be measured quantitatively using a synthetic colorimetric substrate and a spectrophotometer. The amount of APN released was found to be dose-dependent and a reliable measure of the potency of various Bt samples.

Using this assay, we have found all insecticidal proteins tested to date induce APN release, whereas heat-denatured or inactive Bt samples do not. Furthermore, this highly sensitive assay can be used to evaluate the activity of crude Bt culture samples grown on nutrient agar plates using very small sample aliquots. The major advantages of this technique over the traditional bioassay is that it is fast (results can be obtained in hours instead of days) and highly sensitive. The sensitivity is such that it can detect the effects of sublethal doses of Bt samples. This assay overcomes a problem with the conventional assay system, when insects challenged with sublethal doses recover and produce difficulties in obtaining consistent dose-response curves.