Chapter 15-Microbial Insecticides

Insecticidal toxin of *Bacillus thuringiensis*

Baculoviruses as biocontrol agents

Insecticidal toxin of *Bacillus thuringiensis*

- Current market for pesticides is $20 billion/year
- *B. thuringiensis* is a soil bacterium that produces a toxin (Bt toxin or Cry) that kills certain insects
- The Bt toxin or Cry is produced when the bacteria sporulates and is present in the parasporal crystal
- Several different strains and subspecies of *B. thuringiensis* exist and produce different toxins that kill specific insects (Table 15.1)

Table 15.1 Some properties of the insecticidal toxins from various strains of *B. thuringiensis*

Strain/subsp.	Protein size	Target Insects	Cry #
berliner	130-140 kDa	Lepidoptera	CryI
kurstaki KTP, HD1	130-140 kDa	Lepidoptera	CryI
entomocidus 6.01	130-140 kDa	Lepidoptera	CryI
aizawai 7.29	130-140 kDa	Lepidoptera	CryI
aizawai IC 1	135 kDa	Lepidoptera, Diptera	CryII
kurstaki HD-1	71 kDa	Lepidoptera, Diptera	CryII
tenebrionis (sd)	66-73 kDa	Coleoptera	CryIII
morrisoni PG14	125-145 kDa	Diptera	CryIV
israelensis	68 kDa	Diptera	CryIV

The Cry protein: mode of action

- The Cry protein is made as an inactive protoxin
- Conversion of the protoxin (e.g., 130 kDa) into the active toxin (e.g., 68 kDa) requires the combination of a slightly alkaline pH (7.5-8) and the action of a specific protease(s) found in the insect gut
- The active toxin binds to protein receptors on the insect gut epithelial cell membrane
- The toxin forms an ion channel between the cell cytoplasm and the external environment, leading to loss of cellular ATP and insect death

Isolation & genetic engineering of Cry genes

- The Cry (or protoxin) genes are encoded by plasmid DNA, not by chromosomal DNA in *B. thuringiensis*
- Cry genes were expressed in *B. thuringiensis* under the control of the p^{tet} promoter (rather than its sporulation-specific promoter) and provided increase yield
- Constructs have also been produced to enhance toxin action and/or expand its specificity

A potential problem with Cry: development of insect resistance (and how to prevent it)

- Production of hybrid Bt toxins
- Stacking of Bt toxin genes
- Use of Bt toxins in combination with other insecticidal proteins such as chitinase and Cyt1A
- In plants, the planting of crop buffer zones with non-genetically engineered Bt plants to maintain an insect susceptible population

Baculoviruses as biocontrol agents

- Baculoviruses are rod-shaped, double stranded DNA viruses that can infect and kill a large number of different invertebrate organisms
- Baculoviruses have limited host ranges and generally do not allow for insect resistance to develop
- Slow killing of target insects occurs
- In order to speed killing (enhance effectiveness), several genes can be expressed in the baculovirus including diuretic hormone, juvenile hormone esterase, Bt toxin, scorpion toxin, mite toxin, wasp toxin, and a neurotoxin (see Table 15.6)