

Creating Value Through Technology and People



Quality of *Bacillus thuringiensis*

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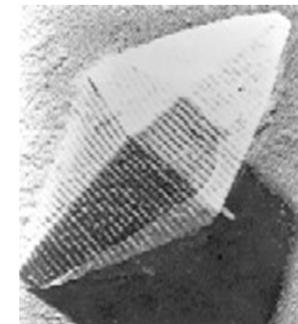
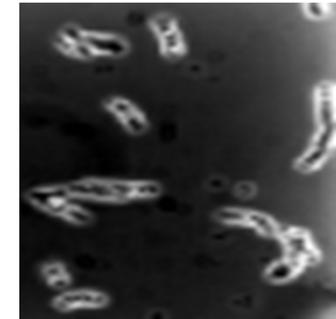
ABIM, Oct 26, 2010





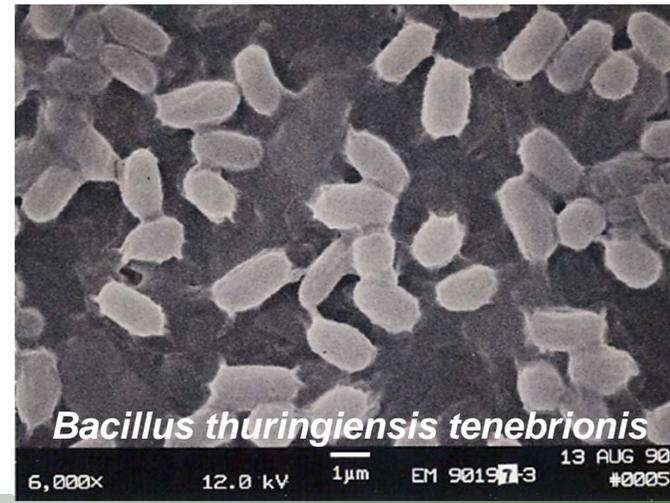
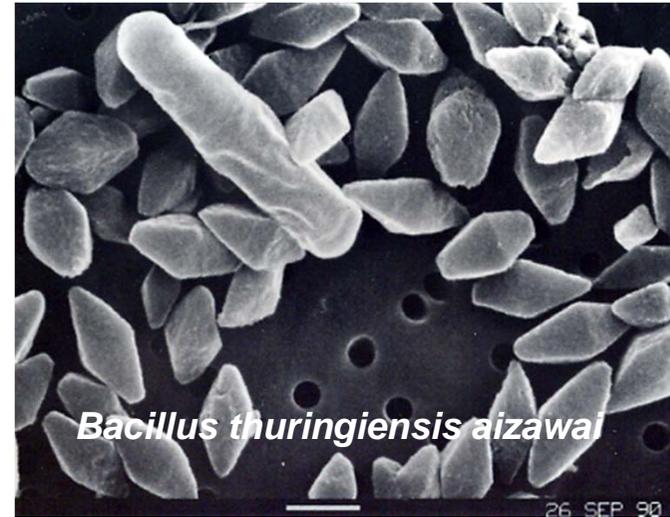
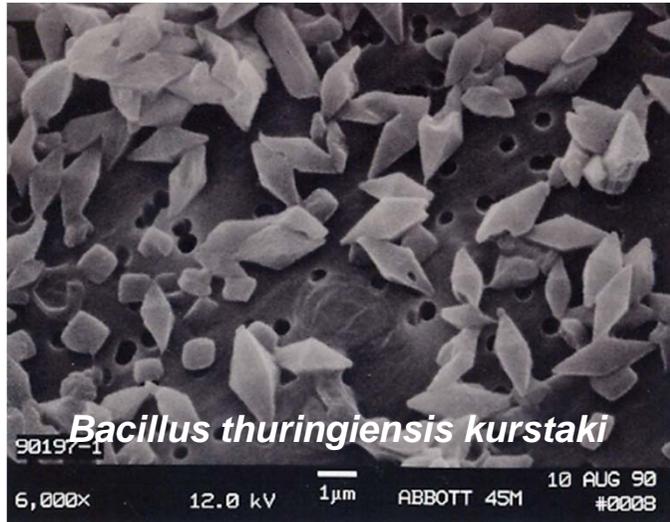
Bacillus thuringiensis (Bt)

- Bt-based insecticides are complex entities
- Using the science and art of fermentation, Bt strains produce a mixture of specific insecticidal proteins as active ingredient
- Bt mode of action is unique and different from any chemical insecticide, with commercially proven efficacy for over 40 years.
- Bt biological insecticides are used in IPM, Organic and Low Residue insect control



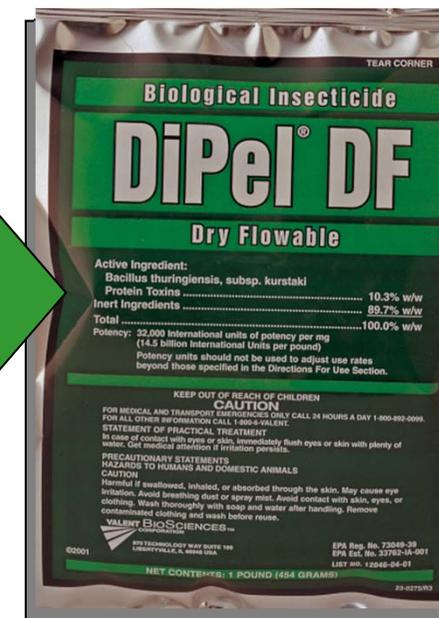
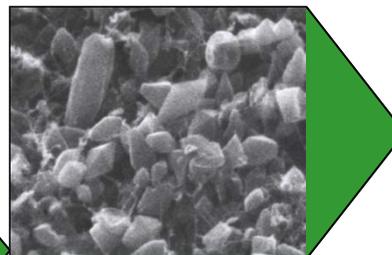


B.t. Insecticidal Crystal Proteins





Bt Manufacturing at VBC



From large scale (100 000 L) fermentation to downstream processing, manufacturing is geared to producing and preserving the best Bt active ingredient mixture at the most economical cost.





Manufacturing Requirements

Regulatory Agencies provide the framework →

→ Manufacturer sets the standards

i.e. US EPA approves the product AND the process

- ✓ All tox/path and environmental tests related to process
- ✓ Processes carried out as filed with EPA
- ✓ Batch records shipping, inventory, disposal records kept

Valent BioSciences sets it's own high standards

- ✓ Frequent in-process sampling for quality
- ✓ Potency checks after each major process step
- ✓ On-site insect bioassay facilities
- ✓ Decades history with Btk and Bta standards





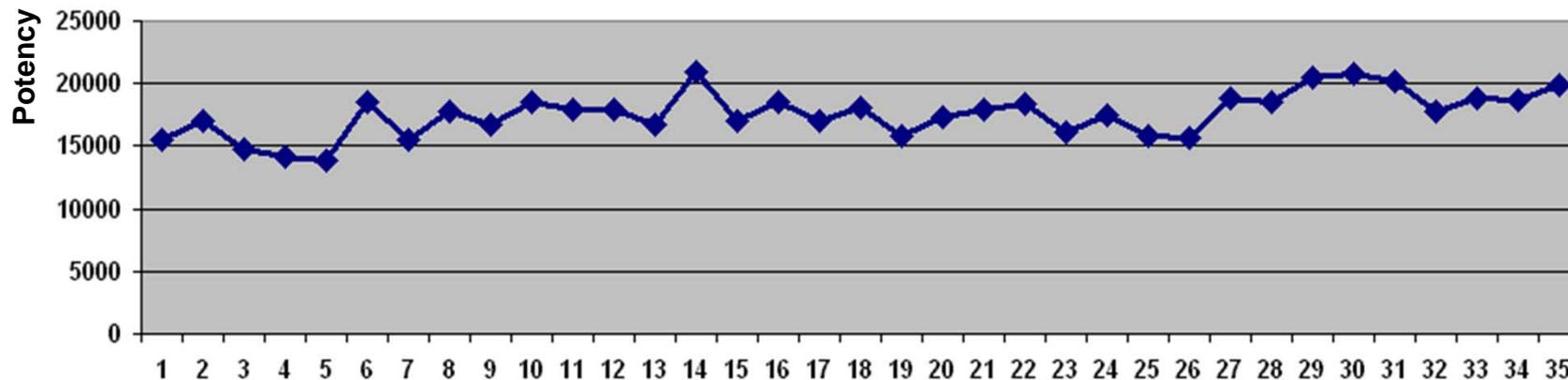
Commercial QC of Bt products

XenTari®	
Insetticida biologico in microgranuli idrodispersibili selettivamente attivo sulle larve di lepidotteri	
Composizione:	
<i>Bacillus thuringiensis</i> subsp. <i>aizawai</i> , sierotipo H7 (Potenza 15.000 U.I./mg di formulato).....	g 10
Coformulanti ed inerti	q.b. g 100
a	
Contiene <i>Bacillus thuringiensis</i> subsp. <i>aizawai</i> , sierotipo H7 può provocare una reazione allergica	
ATTENZIONE MANIPOLARE CON PRUDENZA	
	Valent BioSciences una Divisione di Sumitomo Chemical Agro Europe S.A. Parc d'Affaires de Crécy 2 Rue Claude Chappe 69370 Saint Didier au Mont d'Or – Francia

Commercially, bioassay is the basic QC method.

VBC formulates at least at the labeled potency.

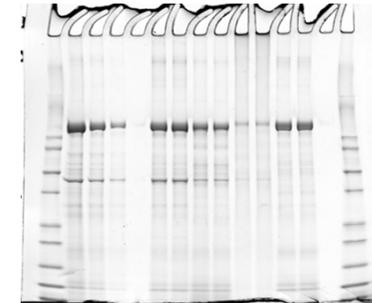
Example: Potency of XenTari® on cabbage looper, *Trichoplusia ni*, is 15 000 IU/mg product. Below are QC bioassay results from 35 large scale XenTari® production runs over several years.



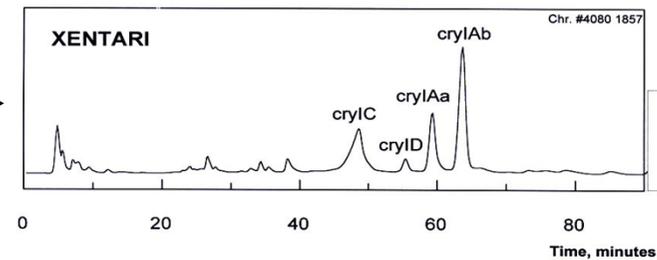


Secondary Analytical Methods for Bt Products

1. SDS-PAGE (electrophoresis method) to quantify Bt toxin proteins

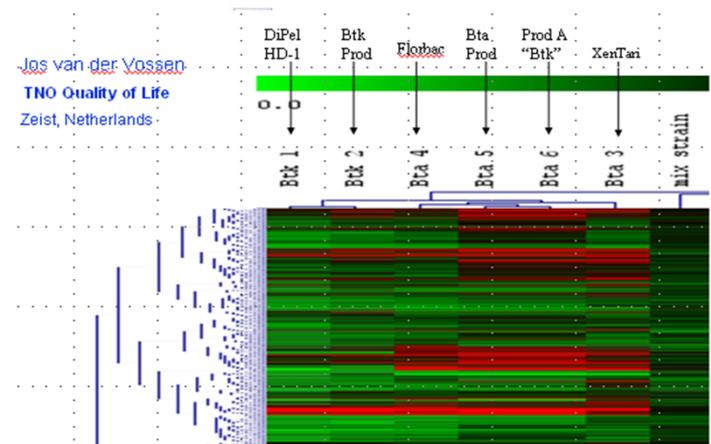


2. HPLC (liquid chromatography method) to separate and quantify individual Bt toxin proteins



3. Gene profiling of Bt toxins

4. Genomotyping (cooperation with TNO, Zeist, the Netherlands)





Large Variability in Bt Quality, Asian Market

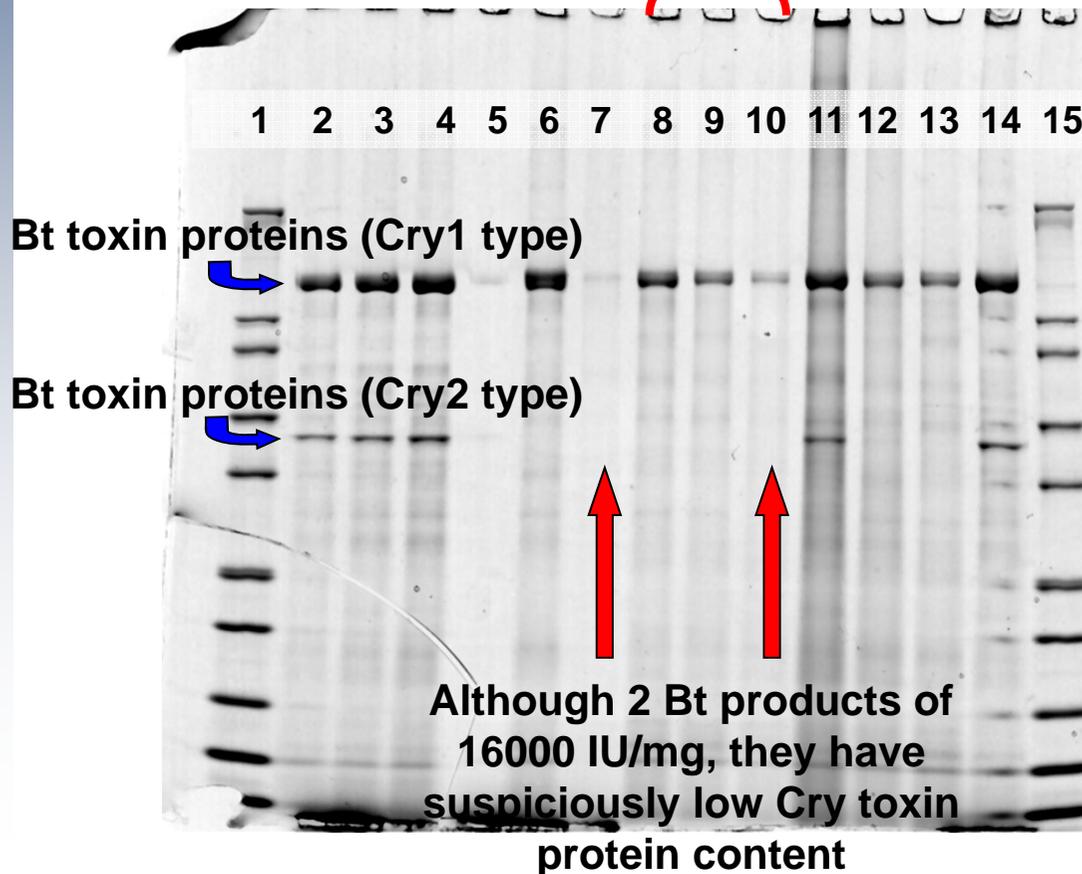
Clearly labeled as Btk
but no Cry2 present

SDS-PAGE analysis

(LC50 in µg/ml on cabbage looper)

Lane:

1. Molecular Weight Marker
2. DiPel® Standard 0.7µg
3. DiPel® Standard 2.1µg
4. DiPel® Standard 4.1µg
5. no sample Blank lane
6. XenTari® (Control)
7. Product 1 SE Asia (LC50: 73.6)
8. Product 2 SE Asia (LC50: 38.8)
9. Product 3 SE Asia (LC50: 64.9)
10. Product 4 SE Asia (LC50: 45.3)
11. Product 5 SE Asia (LC50: 74.4)
12. Product 6 SE Asia (LC50: 78.7)
13. Product 7 SE Asia (LC50: 26.5)
14. DiPel® DF (Control) (LC50: 14.3)
15. Molecular Weight Marker

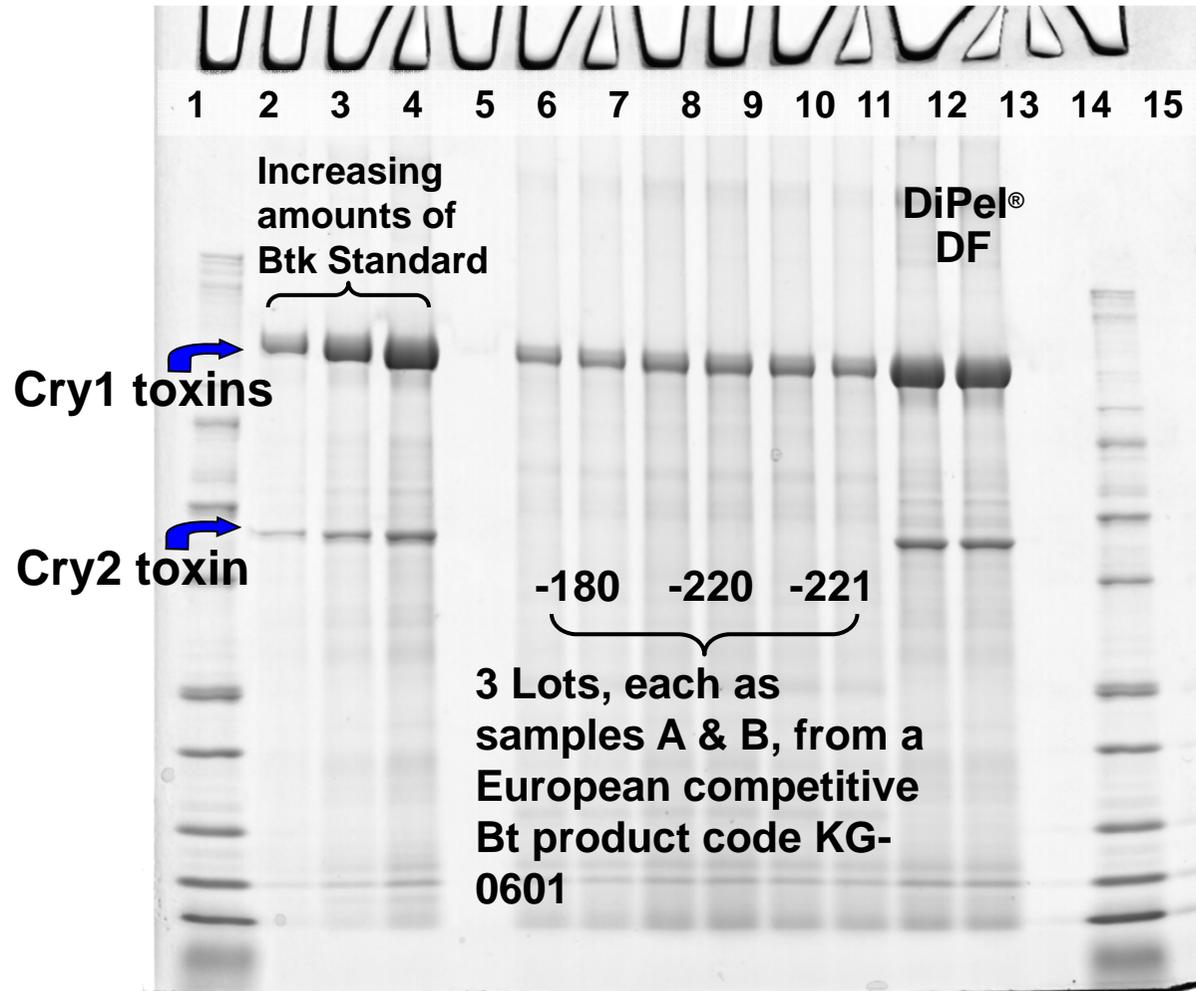


SE Asia Products 1 and 4 were labeled 16000 IU/mg, others at 32000 IU/mg (except XenTari)





SDS-PAGE analysis of a competitive Bt product





DiPel® DF compared to competitive Bt product



Field Rep: Michael Schröder
Investigator: Germán Martínez

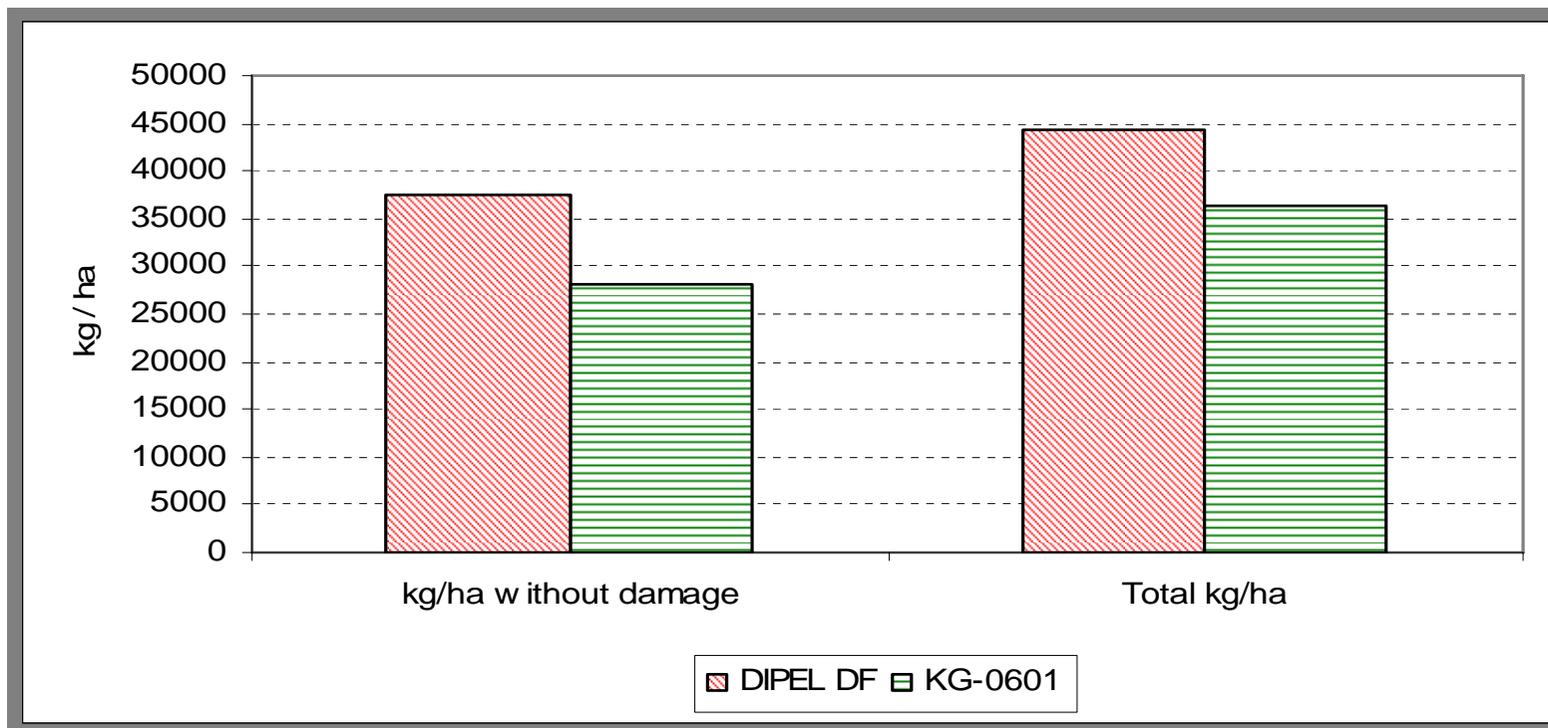
Crop: Industry tomato (organic farming)
Pest: *Helicoverpa armigera*
Application Date: 5 applications on 20/07, 04/08, 10/08, 18/08, and 01/09/2006 with a field boom, 600 – 1000 L/ha, 1 kg/ha

Harvest Date: 20 + 21/09/2006
Plot size: > 1 ha
Site: Finca Huescar, Granada
Country: Spain





Tomato Yield in kg / ha, DiPel® DF vs Competitive Bt



	Kg Tomatoes per ha without damage	Total Kg of Tomatoes per ha
DIPEL DF	37 450	44 441
Competitive Bt	28 206	36 231





Conclusions

1. **Worldwide and within Europe significant differences in quality of Bt products exist. In Asia and Latin America we have found Bt illegally “enhanced” with a chemical insecticide. In Australia and Europe, we have found Bt products with very little toxin protein, and corresponding very low potencies.**
2. **Maintaining the quality of Bt products is left up to the manufacturer. As bio-pesticide manufacturers, we should work together to ensure a certain level of quality based on simple guidelines.**
 - a) repercussions for companies that ameliorate Bt with chemical insecticides
 - b) certain minimum levels of Bt toxin protein weight in the product based on label potency



Thank you

Annual Biocontrol Industry
Meeting Lucerne

