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Persistence of Bt Bacillus thuringiensis Cry1Aa toxin in various soils determined by physicochemical reactions

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Insecticidal Cry proteins from the soil bacterium, Bacillus thuringiensis (Bt) are produced by a class of genetically modified (GM) crops, and released into soils through root exudates and upon decomposition of residues. In contrast to the protoxin produced by the Bacillus, the protein produced in GM crops does not require activation in insect midguts and thereby potentially looses some of its species specificity. Although gene transfer and resistance emergence phenomena are well documented, the fate of these toxins in soil has not yet been clearly elucidated. Cry proteins, in common with other proteins, are adsorbed on soils and soil components. Adsorption on soil, and the reversibility of this adsorption is an important aspect of the environmental behaviour of these toxins. The orientation of the molecule and conformational changes on surfaces may modify the toxicity and confer some protection against microbial degradation. Adsorption will have important consequences for both the risk of exposition of non target species and the acquisition of resistance by target species.

We have adopted different approaches to investigate the fate of Cry1Aa in soils and model minerals. In each series of experiments we endeavoured to maintain the protein in a monomeric form (pH above 6.5 and a high ionic strength imposed with 150 mM NaCl).

The adsorption and the desorbability of the Cry1Aa Bt insecticidal protein were measured on two different homoionic clays: montmorillonite and kaolinite. Adsorption isotherms obtained followed a low affinity interaction for both clays and could be fitted using the Langmuir equation. Binding of the toxin decreased as the pH increased from 6.5 (close to the isoelectric point) to 9. Maximum adsorption was about 40 times greater on montmorillonite (1.71 g g-1) than on kaolinite (0.04 g g-1) in line with the contrasting respective specific surface areas of the minerals. Finally, some of the adsorbed toxin was desorbed by water and more, about 36 %, by high pH buffers, indicating that it was not extremely tightly bound. Moreover, the toxin was easily and quasi-completely desorbed using zwiterrionic and non-ionic detergents.

We have compared the persistence of Cry1Aa on various soils over several weeks varying microbial activity (inhibition or activation). Neither physical nor chemical inhibition of microbial activity led to enhanced persistence of the protein in soil. Stimulation of microbial activity did not accelerate loss of detectable protein. These findings suggest that loss of protein in soil is not determined by microbial breakdown. Chemical fixation and conformational changes may contribute to the observed trends. Hydrophobic interactions with soil organo-mineral surfaces may play an important role in both the adsorption and subsequent changes in conformation of the protein.